

**BEST AVAILABLE COPY**

GB 9515478.7

(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

**EP 1 231 264 A1**

(12)

**EUROPEAN PATENT APPLICATION**

published in accordance with Art. 158(3) EPC

(43) Date of publication:

14.08.2002 Bulletin 2002/33

(51) Int Cl.7: **C12N 9/12, C12N 15/54**

(21) Application number: **00951548.7**

(86) International application number:

**PCT/ES00/00308**

(22) Date of filing: **04.08.2000**

(87) International publication number:

**WO 01/11027 (15.02.2001 Gazette 2001/07)**

(84) Designated Contracting States:

**AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU  
MC NL PT SE**

Designated Extension States:

**AL LT LV MK RO SI**

(72) Inventors:

- **MOSCAT GUILLEN, Jorge,**  
Centro de Biol. Molecular  
28049 Cantoblanco (ES)
- **DIAZ-MECO CONDE, Maria Teresa**  
Centro de Bio. Mol.  
28049 Cantoblanco (ES)

(30) Priority: **06.08.1999 EP 99500139**

(71) Applicant: **Glaxo Wellcome, S.A.**  
**28760 Tres Cantos (Madrid) (ES)**

(74) Representative: **Stott, Michael John**  
**GlaxoSmithKline**  
Corporate Intellectual Property (CN9.25.1)  
980 Great West Road  
Brentford, Middlesex TW8 9GS (GB)

(54) **NEW PROTEIN**

(57) An isolated human BLIP protein or a variant thereof.

**EP 1 231 264 A1**

**Description****Field of the Invention**

5 [0001] The present invention relates to human lambda interacting proteins involved in Nuclear Factor  $\kappa$ B (NF- $\kappa$ B) activation and also, but not exclusively, to kinase-inactive mutants of such proteins; nucleotide sequences encoding such proteins and mutant proteins; and expression vectors, cell lines, antibodies, screening methods, compounds, methods of production and methods of treatment related to the proteins.

**Background of the Invention**

10 [0002] The atypical Protein Kinase C (PKC) isoforms ( $\lambda$ PKC and  $\zeta$ PKC) are necessary for basic cellular functions such as proliferation and survival. Studies have demonstrated that the atypical PKCs (aPKCs) are stimulated by TNF $\alpha$  and are required for the activation of NF- $\kappa$ B by this cytokine, through a mechanism that likely involves the phosphorylation of I $\kappa$ B through the activation of I $\kappa$ B kinase  $\beta$ .

15 [0003] The transcription factor NF- $\kappa$ B plays a critical role in a number of cell functions including key inflammatory and immune responses. Nuclear factor- $\kappa$ B is a ubiquitously expressed multisubunit transcription factor activated in several cell types by a diverse group of inflammatory agents such as TNF $\alpha$ , IL-1 $\beta$ , bacterial endotoxin, and RNA viruses. NF- $\kappa$ B is composed of dimers of different members of the Rel protein family. The classical form of NF- $\kappa$ B is an heterodimer of p50 and p65 (Rel A) (Baeurle and Henkel, 1994; Baldwin, 1996; Thanos and Maniatis, 1995); this is sequestered in the cytosol by I $\kappa$ B which prevents its nuclear translocation and activity.

20 [0004] Upon cell stimulation by inflammatory cytokines such as TNF $\alpha$  or IL-1, I $\kappa$ B is phosphorylated, triggering the ubiquitination and subsequent degradation of I $\kappa$ B through the proteasome pathway (Verma et al., 1995). These events release NF- $\kappa$ B which translocates to the nucleus where it activates several genes (Baeurle and Henkel, 1994; Baldwin, 1996; Thanos and Maniatis, 1995; Verma et al., 1995).

25 [0005] The identification of the kinase responsible for the signal-induced phosphorylation of I $\kappa$ B has been a matter of research. Several groups have identified and cloned two I $\kappa$ B kinase activities (IKK $\alpha$  and IKK $\beta$ ) that phosphorylate residues 32 and 36 of I $\kappa$ B $\alpha$ , and whose activity is potentially stimulated by TNF and IL-1 (DiDonato et al., 1997; Mercurio et al., 1997; Réginer et al., 1997; Woronicz et al., 1997; Zandi et al., 1997). The IKKs bind NIK (NF $\kappa$ B-inducing kinase), a member of the MAPKKK family that interacts with TRAF2 (Malinin et al., 1997), thus linking I $\kappa$ B degradation and NF- $\kappa$ B activation to the TNF receptor complex.

30 [0006] TNF $\alpha$  is a potent activator of the atypical PKCs *in vivo*. It has previously been shown that the atypical PKC isoforms  $\zeta$  (zeta) and  $\lambda$ /I (lambda/iota) play a critical role during NF- $\kappa$ B activation (Díaz-Meco et al., 1996; Folgueira et al., 1996; Sontag et al., 1997). Thus, the blockade of the atypical PKCs by either microinjected pseudosubstrate peptide inhibitors (Dominguez et al., 1993), antisense oligonucleotides (Folgueira et al., 1996), or the transfection of kinase-dead dominant negative mutants of  $\zeta$ PKC or  $\lambda$ /I PKC (Berra et al., 1995; Bjorkoy et al., 1995; Díaz-Meco et al., 1996; Sontag et al., 1997), dramatically impairs NF- $\kappa$ B activation.

35 [0007] However, the mechanisms whereby the atypical PKCs participate in the NF- $\kappa$ B activation pathway remained unclear. Because  $\zeta$ PKC is unable to directly phosphorylate I $\kappa$ B (Díaz-Meco et al., 1994), it is possible that the signals generated by the stimulation of the atypical PKCs could be mediated by the novel IKKs. In this regard, it has recently been demonstrated that the atypical PKCs bind to the IKKs *in vitro* and *in vivo* (Lallena et al., 1999). Overexpression of these PKCs positively modulates IKK $\beta$  but not IKK $\alpha$  activity, whereas the transfection of their respective dominant negative mutants severely impairs the activation of IKK $\beta$  but not of IKK $\alpha$  in TNF $\alpha$ -stimulated cells (Lallena et al., 1999). In addition, recombinant active atypical PKC dramatically stimulates *in vitro* the IKK $\beta$  -- but not IKK $\alpha$  -- activity from unstimulated cells (Lallena et al., 1999). Collectively these results demonstrate a critical role of the atypical PKCs in the NF- $\kappa$ B pathway through the regulation of IKK $\beta$  activity.

40 [0008] In contrast to the classical or novel isoforms of PKCs, the atypical PKCs are insensitive to phorbol esters or diacylglycerol, but are activated by other important lipid second messengers such as phosphatidylinositol 3,4,5-P<sub>3</sub> (Nakanishi et al., 1993), and ceramide (Lozano et al., 1994). Although the products of PI 3-kinase activate the aPKCs (Nakanishi et al., 1993; Akimoto et al., 1996), they also stimulate other kinases such as  $\epsilon$ PKC,  $\theta$ PKC, PRK1 or c-Akt (Díaz-Meco et al., 1996). Ceramide activates both aPKCs (Lozano et al., 1994; Müller et al., 1995) but also stimulates the ceramide-activated protein kinase, among other possible targets. This suggests that the different lipid mediators are not selective for a given PKC.

45 [0009] It has now been discovered that a novel lambda interacting protein (termed BLIP herein) interacts with  $\lambda$ /I PKC in a TNF $\alpha$ -dependent manner. The ectopic expression of wild-type BLIP is sufficient to activate NF- $\kappa$ B in a  $\lambda$ /I PKC dependent manner, whereas a BLIP kinase-inactive mutant is dominant negative. Human and other mammalian BLIP proteins are useful in screening methods for the identification and development of novel pharmaceutical agents, including agonists and antagonists of the proteins, which may be used to increase or decrease the activation of NF $\kappa$ B

(compared to activation which would occur in an untreated control cell).

[0010] Accordingly, it is an object of the present invention to provide isolated human BLIP proteins and DNA encoding such proteins. Other objects of the present invention will become apparent from the following detailed description thereof.

## Summary of the Invention

[0011] According to one embodiment of the present invention there is provided an isolated human BLIP protein or a variant thereof. In a particularly preferred aspect of the invention the protein has an amino acid sequence as provided in **Figure 2A** (SEQ ID NO:4).

[0012] A further aspect of the present invention is a kinase-dead mutant of a BLIP protein.

[0013] A further aspect of the present invention is an isolated human LIP protein or a variant thereof. In a particularly preferred aspect of the invention the protein has an amino acid sequence of SEQ ID NO:6.

[0014] According to another aspect of the invention there is provided an isolated nucleotide sequence encoding a BLIP protein or a variant thereof, or a nucleotide sequence which is complementary thereto. Preferably the nucleotide sequence is a cDNA sequence. Particularly preferably, the nucleotide sequence is or comprises SEQ ID NO:3.

[0015] According to another aspect of the invention there is provided an isolated nucleotide sequence encoding a LIP protein or a variant thereof, or a nucleotide sequence which is complementary thereto. Preferably the nucleotide sequence is a cDNA sequence. Particularly preferably, the nucleotide sequence is or comprises SEQ ID NO:6.

[0016] According to another aspect of the invention there is provided an isolated DNA molecule having a sequence selected from the group consisting of: (a) isolated DNA which encodes the BLIP protein of **Figure 2A** (SEQ ID NO: 4); (b) isolated DNA which hybridizes to isolated DNA of (a) above under conditions represented by a wash stringency of 0.3M NaCl, 0.03M sodium citrate, and 0.1% SDS at 60°C, which is at least 65% homologous to the isolated DNA of (a) above, and which encodes a BLIP protein; and (c) isolated DNA having a sequence complementary to (a) - (c), above.

[0017] According to another aspect of the invention there is provided an expression vector comprising a nucleic acid sequence as referred to above. According to another aspect of the invention there is provided a nucleic acid construct having a promoter and a heterologous nucleic acid operably linked to said promoter, wherein said heterologous nucleic acid is a nucleic acid molecule as given above.

[0018] According to another aspect of the invention there is provided a stable cell line comprising a recombinant DNA sequence or an expression vector as referred to above, capable of expressing the encoded a protein. Preferably the cell line is a modified HEK293 or HeLa cell line.

[0019] According to another aspect of the invention there is provided a polyclonal or monoclonal antibody that specifically binds to a BLIP protein as given above.

[0020] According to another aspect of the invention there is provided an antisense oligonucleotide complementary to a nucleic acid as given above and having a length sufficient to hybridize thereto under physiological conditions; along with DNA encoding such an antisense oligonucleotide; and a nucleic acid construct having a promoter and a heterologous nucleic acid operably linked to said promoter, wherein the heterologous nucleic acid is a DNA encoding such an antisense oligonucleotide.

[0021] According to another aspect of the invention there is provided a method of inhibiting NF- $\kappa$ B activation in a cell which comprises providing to the cell an inhibitor of BLIP-related NF- $\kappa$ B activation, in an amount effective to inhibit NF- $\kappa$ B activation therein. The cell may be provided in any suitable form, such as in in vitro culture. The providing step may be carried out by any suitable means, such as by delivering the inhibitory compound into the cell or by delivering into the cell a nucleic acid encoding and expressing an inhibitory protein. The cell is preferably a mammalian cell and more preferably a human cell.

[0022] A further aspect of the present invention is the use of a BLIP kinase-dead mutant, or a LIP protein, as an inhibitor of NF- $\kappa$ B activation in a cell, or as an anti-cancer agent or adjuvant.

[0023] According to another aspect of the invention there is provided a method for screening compounds for the ability to modulate (enhance or inhibit) BLIP-dependent activation of NF- $\kappa$ B. Such methods comprise contacting a test compound with a BLIP protein as referred to above and detecting modulating activity or inactivity.

[0024] According to another aspect of the invention there is provided a compound that modulates BLIP-dependent NF- $\kappa$ B activity, identifiable by the methods referred to above.

[0025] According to another aspect of the invention there is provided a method of treatment or prophylaxis of a disorder that is responsive to modulation of BLIP-dependent NF- $\kappa$ B activation, in a mammalian subject, that comprises administering to said patient an effective amount of a compound that modulates (inhibits or enhances) BLIP-dependent NF- $\kappa$ B activation. Preferably the disorder is selected from neoplastic, inflammatory and immune disorders.

[0026] According to a further aspect of the invention there is provided use of a compound that modulates of BLIP-dependent NF- $\kappa$ B activation in a method of formulating a medicament for treatment or prophylaxis of a disorder that

is responsive to modulation of BLIP-dependent NF- $\kappa$ B activation.

#### Brief Description of the Figures

**[0027]** The present invention will be further described by way of example and with reference to the following figures:

**Figure 1** graphs the effect of overexpression of LIP on NF- $\kappa$ B activation; increasing expression of LIP decreased TNF $\alpha$ -induced  $\kappa$ B-dependent reporter activity.

**Figure 2A** shows the amino acid sequence of the human BLIP protein (SEQ ID NO:4).

**Figure 2B** is a schematic representation of BLIP showing the FRAP/ATM PI 3-kinase-like domain and the LIP region.

**Figure 3A** is an alignment of the PI 3-kinase domain of human BLIP with that of FRAP. Asterisks indicate amino acids that are identical in both aligned proteins; the conserved critical residues that confer catalytic activity to the protein are boxed.

**Figure 3B** is an alignment of the PI 3-kinase domain of human BLIP with that of ATM. Asterisks indicate amino acids that are identical in both aligned proteins; the conserved critical residues that confer catalytic activity to the protein are boxed.

**Figure 4A** graphs the effects of BLIP on NF- $\kappa$ B activation in cultures of human HEK293 cells. The HEK293 cells were transfected with a  $\kappa$ B-dependent luciferase reporter plasmid and either: a negative control vector (first bar); increasing concentrations of an expression plasmid for wild-type BLIP (BLIP; second - sixth bars); increasing concentrations of an expression plasmid for kinase-inactive BLIP (BLIP<sup>MUT</sup>; seventh - eleventh bars); or an RIP expression vector (positive control; final bar). After 24 hours the levels of luciferase expression were determined. Results are the mean  $\pm$  SD of three independent experiments with incubations in duplicate.

**Figure 4B** graphs the role of BLIP on NF- $\kappa$ B activation in cultures of HEK293 cells. Cells were transfected with a  $\kappa$ B-dependent luciferase reporter plasmid and either: a negative control vector (white bars), an expression plasmid for wild type  $\lambda$ iPKC ( $\lambda$ iPKC; hatched bars), or an expression plasmid for kinase-inactive  $\lambda$ iPKC ( $\lambda$ iPKC<sup>MUT</sup>; solid bars). Luciferase activity in cells without BLIP (control) and with increasing concentrations of wild-type BLIP (BLIP) was assessed after 24 hours. Results are the mean  $\pm$  SD of three independent experiments with incubations in duplicate.

**Figure 5** shows the percentage of HeLa cells with nuclear p65 after transfection with a vector for an HA-tagged version of BLIP<sup>MUT</sup>. Twenty-hours post-transfection, cells were either untreated (control; white bars) or stimulated for 20 min with TNF $\alpha$  (30 ng/ml; solid bars). Analysis was by immunofluorescence confocal microscopy with monoclonal anti-HA antibody 12CA5 and a goat polyclonal anti-p65 antibody. The graph shows the mean  $\pm$  SD of several fields (300 cells) from three independent experiments.

**Figure 6** provides the nucleotide sequence (SEQ ID NO:3) of DNA encoding the BLIP protein of SEQ ID NO:4.

#### Detailed Description of the Invention

**[0028]** The present inventors have isolated and characterized a novel LIP isoform, herein termed BLIP (for "big LIP") (SEQ ID NO:4). BLIP interacts with  $\lambda$ iPKC in a TNF $\alpha$ -dependent manner, and activates NF- $\kappa$ B in a  $\lambda$ iPKC-dependent manner. Of functional relevance, BLIP has a domain that is highly homologous to the PI 3-kinase-like region of FKBP-rapamycin associated protein (FRAP) and Ataxia Telangiectasia Mutated (ATM) protein (see **Figs. 3A** and **3B**). Interestingly, the ectopic expression of wild-type BLIP is sufficient to activate NF- $\kappa$ B in a  $\lambda$ iPKC dependent manner, whereas a BLIP kinase-inactive mutant is dominant negative. This is the first evidence of the involvement of a FRAP/ATM-like molecule in the activation of NF- $\kappa$ B. NF- $\kappa$ B has been shown to be relevant in cell proliferation and apoptosis (Baldwin, 1996; Lenardo and Baltimore, 1989). The present inventors further expanded the known sequence of the human LIP protein, and determined that the LIP protein inhibits NF- $\kappa$ B dependent transcription when expressed in a cell.

**[0029]** The atypical PKCs are regulated by protein regulators. Compared to the amino acid sequences of classical or novel PKCs, the sequences of the atypical PKC subfamily differ in the regulatory domain. The identification of selective modulators for each PKC isotype will assist in understanding the function and regulation of the different PKC

isoforms. A protein named LIP has been identified as a selective regulator of  $\lambda/\mu$ PKC (Diaz-Meco et al., 1996). LIP binds to the zinc finger of  $\lambda/\mu$ PKC but does not bind to  $\alpha$ PKC,  $\epsilon$ PKC or even to the highly related  $\zeta$ PKC (Diaz-Meco et al., 1996). Interestingly, overexpression of a fragment of LIP (the R4 fragment) that is sufficient to interact with  $\lambda/\mu$ PKC is capable of stimulating a  $\kappa$ B-dependent promoter activity (Diaz-Meco et al., 1996).

5 [0030] Previously it was reported that expression of a minimal C-terminal region of LIP (named R4 in Diaz-Meco et al., 1996) that bound  $\lambda/\mu$ PKC was also sufficient to activate NF- $\kappa$ B (Diaz-Meco et al., 1996). However, the present inventors determined that the overexpression of full-length LIP does not activate NF- $\kappa$ B, and in fact inhibits  $\kappa$ B-dependent gene transcription (see Example 4).

10 [0031] LIP was initially identified by the two-hybrid system in yeast. An isolated cDNA of LIP encoding a protein of 713 amino acids with a predicted molecular weight of 79.7 kDa is described by Diaz-Meco et al. (1996). The present inventors determined the sequence of LIP to be 779 amino acids (SEQ ID NO:6), and observed that LIP was expressed in testicle and heart tissues but not in other tissues investigated (liver, ovary, spleen, thymus, brain, lung and kidney). However, a band that reacted with the anti-LIP antibody and having a molecular weight higher than 200 kDa was unexpectedly detected (results not shown).

15 [0032] The expression of LIP and the newly identified band was then determined in two different human cell lines (HEK293 and HeLa cells). See Example 2. It was observed that both bands were detectable in both cell lines although the proportion varied, with the high molecular weight protein (BLIP) more abundant in HEK293 than in HeLa cells (results not shown).

20 [0033] The present inventors assessed whether BLIP could bind to  $\lambda/\mu$ PKC in vivo (Example 3), using HEK293 cells that were either untreated (control) or stimulated with TNF $\alpha$  for various times. Under resting conditions there was little or no association of BLIP or LIP to  $\lambda/\mu$ PKC (data not shown). However, upon TNF $\alpha$  stimulation there was a rapid and sustained interaction of BLIP with  $\lambda/\mu$ PKC.

[0034] To further investigate whether BLIP was physiologically relevant in the  $\alpha$ PKC/NF- $\kappa$ B pathway, the present inventors next cloned and molecularly characterized BLIP (Example 5). BLIP is a protein of 2392 amino acids (Fig. 2A, SEQ ID NO:4); the region encompassing amino acids 881-1209 of BLIP displays a high degree of homology with the PI 3-kinase domains of FRAP (SEQ ID NO:1) and ATM (SEQ ID NO:2) (Figs. 3A and 3B). The alignment of the PI 3-kinase-like domains of BLIP, FRAP and ATM indicates that BLIP has kinase activity, as the DXXXXN and DFG motifs that constitute critical residues of the catalytic site conserved in all ATM family members with kinase activity, are also conserved in BLIP (see Figs. 3A and 3B).

30 [0035] The present inventors investigated whether the FRAP/ATM-like kinase domain of BLIP plays a role in NF- $\kappa$ B activation (Example 6). HEK293 cells were transfected with a  $\kappa$ B-dependent reporter gene (luciferase) along with increasing concentrations of expression plasmids for BLIP, either wild-type BLIP or a kinase-inactive mutant (BLIP<sup>MUT</sup>). The results (Figure 4A) indicate that the expression of wild-type BLIP (but not a kinase inactive mutant BLIP) is sufficient to activate the  $\kappa$ B-dependent reporter activity to an extent comparable to that of RIP (Receptor Interacting Protein).

35 [0036] Further experiments established that the NF- $\kappa$ B activation effect of BLIP is dependent on  $\lambda/\mu$ PKC (Example 7). Cells were transfected with a  $\kappa$ B-dependent luciferase reporter plasmid and either a negative control vector, an expression plasmid for wild type  $\lambda/\mu$ PKC, or an expression plasmid for kinase-inactive  $\lambda/\mu$ PKC<sup>MUT</sup>. Luciferase activity in cells without BLIP (control) and with increasing concentrations of wild-type BLIP (BLIP) was assessed, and it was seen that the co-expression of wild-type  $\lambda/\mu$ PKC cooperated with BLIP to activate NF- $\kappa$ B, whereas the expression of the kinase-inactive  $\lambda/\mu$ PKC mutant did not. Results are shown in Figure 4B.

40 [0037] The present inventors' work indicates that BLIP is a critical component of the NF- $\kappa$ B pathway, and depends on its own kinase activity and that of  $\lambda/\mu$ PKC to exert its function.

[0038] To demonstrate that BLIP controls the translocation of RelA to the nucleus, cells were transfected with the HA-tagged BLIP mutant expression vector (BLIP<sup>MUT</sup>). Twenty-four hours post-transfection the cells were either stimulated with TNF $\alpha$  or untreated (control). The cells were then analyzed by double immunofluorescence using an anti p65 (RelA) antibody and anti HA-antibody to detect cells that were expressing the construct (data not shown). Analysis of the cells revealed cells transfected with the BLIP mutant had inhibited TNF $\alpha$ -induced translocation of p65 compared to cells transfected with wild-type BLIP (Fig. 5).

45 [0039] The atypical PKCs are involved in the control of NF- $\kappa$ B and p70S6K (Romanelli et al., 1999). Both pathways are critical in the control of inflammatory and immunosuppressor cell function. NF- $\kappa$ B controls a myriad of immune responses and is a potent anti-apoptotic molecule. Inhibition of the atypical PKC kinases blocks the growth and proliferation of cells and initiates a programmed cell death (Berra et al., 1993, 1997; Diaz-Meco et al., 1996; Murray et al., 1997). In addition, the inhibition of  $\alpha$ PKCs leads to a reversion of the transforming phenotype of Ras-transformed cells (Bjorkoy et al., 1995), indicating that inhibitors of the atypical PKCs may have anti-neoplastic effects and would be suitable for screening for anti-cancer therapeutic effects. Consistent with this notion, the atypical PKCs target key intermediaries in the cell signaling cascades involved in cell proliferation. It has been demonstrated that  $\zeta$ PKC as well as  $\lambda/\mu$ PKC control the activation of the MEK-ERK signaling cascade, along with Raf, in the Ras and the PI 3-kinase  $\gamma$  pathways (Diaz-Meco et al., 1994, 1995; Liao et al., 1997; Schönwasser et al., 1998; Takeda et al., 1999).

**[0040]** The present inventors determined that BLIP has a domain highly homologous to the kinase region of FRAP. FRAP is the target of the immunosuppressor molecule rapamycin (Dennis et al., 1999). Rapamycin blocks the activation of p70S6K in T cells which constitutes the molecular basis of its immunosuppressor actions (Dennis et al., 1999). Interestingly, the activity of p70S6K is required and sufficient for the cell cycle progression of T lymphocytes. The BLIP- $\lambda$ iPKC tandem represents an alternate pathway to that of FRAP in the design of potentially novel immunosuppressor molecules.

**[0041]** The results reported herein demonstrate the critical role of BLIP in NF- $\kappa$ B activation, and also demonstrate that the FRAP/ATM-like kinase domain of BLIP is important in NF- $\kappa$ B activation. A kinase-dead mutant of BLIP protein is unable to activate NF- $\kappa$ B and even has a dominant negative activity when transfected in TNF $\alpha$ -activated cells (similar to the effects of the full-length LIP).

**[0042]** While not wishing to be held to a single theory underlying the mechanisms of the LIP and BLIP proteins, the present investigators postulate that the full-length LIP constrains the ability of  $\lambda$ iPKC to activate NF- $\kappa$ B. This constraint is not present when the R4 fragment is used to investigate the activation of NF- $\kappa$ B (Diaz-Meco et al., 1996). It is possible that in the case of BLIP, the putative constraint is relieved by the action of the FRAP/ATM-like kinase activity.

**[0043]** Throughout the present specification and the accompanying claims the words "comprise" and "include" and variations such as "comprises", "comprising", "includes" and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

**[0044]** Amino acid sequences disclosed herein are presented in the amino to carboxy direction, from left to right. The amino and carboxy groups are not presented in the sequence. Nucleotide sequences are presented herein by single strand only, in the 5' to 3' direction, from left to right. Nucleotides and amino acids are represented herein in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by three letter code, in accordance with established usage.

**[0045]** As referred to above, the present invention relates to isolated BLIP and LIP proteins. Sequence information for isolated human BLIP is provided in **Figure 2A** (amino acid; SEQ ID NO:4) and **Fig. 6** (nucleotide; SEQ ID NO:3). Amino acid sequence information for a kinase-inactive mutant of BLIP is provided in SEQ ID NO:5. Sequence information for LIP is provided in SEQ ID NO:6 (amino acid) and SEQ ID NO:7 (nucleotide). In the context of this invention the term "isolated" indicates that the protein or nucleotide molecule is not in its native state, insofar as it has been purified at least to some extent or has been synthetically produced, for example by recombinant methods. The term "isolated" therefore includes the possibility of the molecule being in combination with other biological or non-biological material, such as cells, suspensions of cells or cell fragments, proteins, peptides, expression vectors, organic or inorganic solvents, or other materials where appropriate, but excludes the situation where the protein is in a state as found in nature.

**[0046]** Routine methods known in the art, as further explained in the subsequent experimental section, can be employed to purify and/or synthesise the proteins and nucleotide molecules according to the invention. Such methods are well understood by persons skilled in the art, and include techniques such as those disclosed in Sambrook, J. et al (1), the disclosure of which is included herein in its entirety by way of reference.

**[0047]** The term "BLIP variant" as used throughout the specification and claims refers to other peptides or proteins that retain the same essential functional character of the BLIP protein of SEQ ID NO:4 provided herein, but which vary in amino acid sequence. Variants are also intended to be included within the scope of the invention. For example, peptides or proteins with greater than about 65%, preferably at least 70%, 75%, 80% or 85%, and particularly preferably at least about 90% or even 95% or 98% sequence similarity with SEQ ID NO:4, and which retain BLIP functions, are considered as variants of the BLIP protein. Such variants may include the deletion, modification or addition of single amino acids or groups of amino acids within the protein sequence, as long as the peptide maintains the basic biological functionality of a BLIP protein, i.e., interacts with  $\lambda$ iPKC in a TNF $\alpha$ -dependent manner and activates NF- $\kappa$ B in a TNF $\alpha$ -dependent and  $\lambda$ iPKC-dependent manner. This biological functionality can be assessed by conducting studies as described herein and as known in the art.

**[0048]** The term "BLIP protein" as used herein does not include a protein consisting only of the LIP protein sequence (SEQ ID NO:6) or the R4 fragment (SEQ ID NO:8).

**[0049]** As used herein, a BLIP protein is one having amino acid SEQ ID NO:4, or having amino acid similarity of at least about 65%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or even 99% to SEQ ID NO:4 and having BLIP functions. A protein having BLIP functions is one that interacts with  $\lambda$ iPKC in a TNF $\alpha$ -dependent manner, activates NF- $\kappa$ B in a TNF $\alpha$ -dependent and  $\lambda$ iPKC-dependent manner, and contains a functional kinase domain. The degree of amino acid sequence similarity between proteins can be calculated using various commercially available computer software programs (e.g., BLAST analysis, as is known in the art).

**[0050]** As used herein, a kinase inactive mutant of BLIP is a protein or peptide having substantial sequence similarity to a functional BLIP protein (e.g., sequence similarity of at least about 65%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or even 99%), but having a non-functional kinase domain. One skilled in the art will recognize the PI-3 kinase domain

of a BLIP protein by its sequence. An example of a kinase-inactive BLIP mutant is provided herein by SEQ ID NO:5.

**[0051]** The term "LIP variant" as used throughout the specification and claims refers to other peptides or proteins that retain the same essential functional character of the LIP protein of SEQ ID NO:6 provided herein, but which vary in amino acid sequence. Variants are also intended to be included within the scope of the invention. For example, peptides or proteins with greater than about 90%, 92%, 95% or even 97% or 98% sequence similarity with SEQ ID NO:6, and which retain LIP functions, are considered as variants of the LIP protein. Such variants may include the deletion, modification or addition of single amino acids or groups of amino acids within the protein sequence, as long as the peptide maintains the basic biological functionality of a LIP protein, i.e., inhibits NF- $\kappa$ B dependent transcription. This biological functionality can be assessed by conducting studies as described herein and as known in the art. As used herein, a LIP protein is one having amino acid SEQ ID NO:6, or having amino acid similarity of at least about 90%, 92%, 95%, 97%, 98% or even 99% to SEQ ID NO:6 and having LIP functions. The degree of amino acid sequence similarity between proteins can be calculated using various commercially available computer software programs (e.g., BLAST analysis, as is known in the art).

**[0052]** In general, those skilled in the art will appreciate that minor deletions or substitutions may be made to the amino acid sequences of peptides and proteins of the present invention without unduly adversely affecting the activity thereof. Thus, proteins containing such deletions or substitutions are a further aspect of the present invention. In peptides containing substitutions or replacements of amino acids, one or more amino acids of a sequence may be replaced by one or more other amino acids wherein such replacement does not affect the function of that sequence. Such changes can be guided by known similarities between amino acids in physical features such as charge density, hydrophobicity/hydrophilicity, size and configuration, so that amino acids are substituted with other amino acids having essentially the same functional properties, as is known in the art.

**[0053]** The invention also includes nucleotide sequences that encode BLIP and LIP proteins, mutants, and variants thereof as well as nucleotide sequences which are complementary thereto. The nucleotide sequence may be RNA or DNA including genomic DNA, synthetic DNA or cDNA. Preferably the nucleotide sequence is a DNA sequence and most preferably, a cDNA sequence. (Nucleotide sequence information is provided in **Figure 6** (SEQ ID NO:3); the BLIP protein of SEQ ID NO:4 is encoded by nucleotides 152 - 7327 of SEQ ID NO:3). Nucleotide molecules can be isolated from human cells or synthesised according to methods known in the art, as described by way of example in Sambrook, J. et al (1), the disclosure of which is included herein in its entirety by reference. The nucleotide molecules according to the invention have utility in production of the proteins according to the invention, which may take place *in vitro*, *in vivo* or *ex vivo*. The nucleotide molecules may be involved in recombinant protein synthesis or indeed as therapeutic agents in their own right, utilised in gene therapy techniques. Nucleotides complementary to those encoding BLIP proteins, or antisense sequences, may also be used in gene therapy, such as in strategies for down-regulation of expression of the proteins of the invention.

**[0054]** As used herein, DNA molecules that encode a BLIP protein is intended to encompass natural allelic variations in the DNA molecules. Hybridization conditions which permit other DNA sequences which code for a BLIP protein to hybridize to a DNA sequence as given herein (e.g., SEQ ID NO:3) are, in general, high stringency conditions. For example, hybridization of such sequences may be carried out under conditions represented by a wash stringency of 0.3M NaCl, 0.03M sodium citrate, 0.1% SDS at 60°C or even 70°C in a standard *in situ* hybridization assay. See J. Sambrook et al., *Molecular Cloning, A Laboratory Manual* (2d Ed. 1989)(Cold Spring Harbor Laboratory)). In general, DNA sequences which code for a BLIP protein and hybridize to a DNA sequence of SEQ ID NO:3 disclosed herein will have at least 65%, 70%, 75%, 80%, 85%, 90%, 92% or even 95% nucleotide sequence similarity with SEQ ID NO:3, and encode a protein have BLIP functions. Sequence similarity may be determined using commercially available computer software programs (e.g., BLAST analysis as is known in the art). Isolated DNA of the present invention can be of any species of origin, including mouse, rat, rabbit, cat, porcine, and human, but is preferably of mammalian origin and most preferably of human origin.

**[0055]** As used herein, a DNA molecule encoding a LIP protein is intended to encompass natural allelic variations in the DNA molecules. Hybridization conditions which permit other DNA sequences which code for a LIP protein to hybridize to a DNA sequence as given herein (e.g., SEQ ID NO:7) are, in general, high stringency conditions. For example, hybridization of such sequences may be carried out under conditions represented by a wash stringency of 0.3M NaCl, 0.03M sodium citrate, 0.1% SDS at 60°C or even 70°C in a standard *in situ* hybridization assay. See J. Sambrook et al., *Molecular Cloning, A Laboratory Manual* (2d Ed. 1989)(Cold Spring Harbor Laboratory)). In general, DNA sequences which code for a LIP protein and hybridize to a DNA sequence of SEQ ID NO:3 disclosed herein will have at least 65%, 70%, 75%, 80%, 85%, 90%, 92% or even 95% nucleotide sequence similarity with SEQ ID NO:3, and encode a protein have LIP functions. Sequence similarity may be determined using commercially available computer software programs (e.g., BLAST analysis as is known in the art). Isolated DNA of the present invention can be of any species of origin, including mouse, rat, rabbit, cat, porcine, and human, but is preferably of mammalian origin and most preferably of human origin.

**[0056]** Further, oligonucleotide molecules that code for the BLIP or LIP proteins (or mutants or variants thereof), but

which differ in codon sequence from the foregoing DNA molecules due to the degeneracy of the genetic code, are also an aspect of this invention. The degeneracy of the genetic code, which allows different nucleic acid sequences to code for the same protein or peptide, is well known in the art. Oligonucleotide molecules that code for the same BLIP protein as coded for by the foregoing sequences, but which differ in codon sequence from these due to site directed mutagenesis are yet another aspect of this invention. Site directed mutagenesis techniques are known in the art.

**[0057]** The present invention also includes expression vectors that comprise nucleotide sequences encoding the present proteins, mutants or variants thereof. Such expression vectors are routinely constructed in the art of molecular biology and may for example involve the use of plasmid DNA and appropriate initiators, promoters, enhancers and other elements, such as for example polyadenylation signals which may be necessary, and which are positioned in the correct orientation to allow protein expression. Suitable vectors will be apparent to persons skilled in the art. By way of further example in this regard we refer to Sambrook et al (1), the disclosure of which is included herein in its entirety.

**[0058]** Vectors may be used either to amplify DNA encoding a desired protein and/or to express DNA which encodes a desired protein. The DNA sequence encoding the desired protein is operably linked to suitable control sequences capable of effecting the expression of the protein in a suitable host. DNA regions are operably linked or operably associated when they are functionally related to each other. For example, a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. The need for such control sequences will vary depending upon the host selected and the transformation method chosen. Vectors may include plasmids, viruses (e.g., adenovirus, cytomegalovirus), phage, and integratable DNA fragments (i.e., fragments integratable into the host genome by recombination).

**[0059]** The invention also includes cell lines that have been modified or transfected to express the proteins of the present invention. Such cell lines include transient, or preferably stable higher eukaryotic cell lines (such as mammalian cells or insect cells), lower eukaryotic cells (such as yeast) or prokaryotic cells (such as bacterial cells). It is also possible for the proteins of the invention to be transiently expressed in a cell line, such as for example in a baculovirus expression system.

**[0060]** According to another aspect, the present invention also relates to antibodies (polyclonal or preferably monoclonal antibodies) that specifically bind to the proteins of the present invention (i.e., antibodies which bind to a single antigenic site or epitope on the proteins). Such antibodies are preferably of monoclonal origin. The antibodies are preferably IgG antibodies of any suitable species, such as rat, rabbit, or horse, but are generally of mammalian origin. Fragments of IgG antibodies which retain the ability to specifically bind the proteins of the present invention, such as F(ab')<sub>2</sub>, F(ab'), and Fab fragments, are intended to be encompassed by the term "antibody" herein. See generally E. Harlow and D. Lane, *Antibodies: A Laboratory Manual* (1988) (New York: Cold Spring Harbor Laboratory Press). The antibodies may be chimeric, as described by M. Walker et al., *Molecular Immunol.* 26, 403 (1989). Antibodies that bind to BLIP proteins, but not to shorter LIP proteins, are particularly preferred.

**[0061]** Such antibodies are useful in purification, isolation or screening involving immunoprecipitation techniques and may be used as tools to further elucidate the BLIP protein function, or as therapeutic agents in their own right.

**[0062]** Nucleic acid oligodeoxynucleotides complementary to and capable of hybridizing to the sense strand of a gene, or to an mRNA transcribed from that gene, are antisense oligodeoxynucleotides. When the sense strand of a target gene, or an mRNA, is exposed to its antisense oligodeoxynucleotides, interaction of the two will occur such that the gene will be blocked from transcription or the mRNA blocked from translation. The present invention includes oligomers capable of hybridizing to a portion of a gene (or an mRNA) encoding the BLIP or LIP protein. When such an oligomer is hybridized to the target molecule, production of the protein will be substantially prevented or reduced (compared to that which would occur in the absence of the antisense oligonucleotide). Substantial prevention means that the protein is not produced, or is produced at non-functional levels.

**[0063]** Antisense oligonucleotides (and nucleic acids that express such antisense molecules) may be made in accordance with conventional techniques. See, e.g., U.S. Pat. No. 5,023,243 to Tullis; U.S. Pat. No. 5,149,797 to Pederson et al. The length of the antisense oligonucleotide (i.e., the number of nucleotides therein) is not critical so long as it binds selectively to the intended location; this can be determined in accordance with routine procedures. In general, the antisense oligonucleotide will be from 8, 10 or 12 nucleotides in length up to 20, 30, or 50 nucleotides in length. Such antisense oligonucleotides may be oligonucleotides wherein at least one, or all, of the internucleotide bridging phosphate residues are modified phosphates, such as methyl phosphonates, methyl phosphonothioates, phosphoromorpholidates, phosphoropiperazidates and phosphoramidates. For example, every other one of the internucleotide bridging phosphate residues may be modified as described. In another nonlimiting example, such antisense oligonucleotides are oligonucleotides wherein at least one, or all, of the nucleotides contain a 2' loweralkyl moiety (e.g., C1-C4, linear or branched, saturated or unsaturated alkyl, such as methyl, ethyl, ethenyl, propyl, 1-propenyl, 2-propenyl, and isopropyl). For example, every other one of the nucleotides may be modified as described. See also P. Furdon et al., *Nucleic Acids Res.* 17, 9193-9204 (1989); S. Agrawal et al., *Proc. Natl. Acad. Sci. USA* 87, 1401-1405 (1990); C. Baker et al., *Nucleic Acids Res.* 18, 3537-3543 (1990); B. Sproat et al., *Nucleic Acids Res.* 17, 3373-3386 (1989); R. Walder



and J. Walder, Proc. Natl. Acad. Sci. USA 85, 5011-5015 (1988).

**[0064]** A further aspect of the present invention is screening methods designed to identify compounds that modulate BLIP activation of  $\lambda$ I $\kappa$ B, or that modulate NF- $\kappa$ B activation. By the term "modulation" it is meant that there will be either an increase or a decrease in NF- $\kappa$ B activation in the presence of the compound (compared to that which would be observed in the absence of the compound); such activity generally will result from ligand binding of the compound to BLIP,  $\lambda$ I $\kappa$ B, the tandem of BLIP and  $\lambda$ I $\kappa$ B, or to NF- $\kappa$ B itself.

**[0065]** In general terms, such screening methods involves administering a test compound to a cell and detecting BLIP-related activation of NF- $\kappa$ B, compared to a control cell in which the test compound (or functional BLIP protein) is absent. Activation of NF- $\kappa$ B may be assessed using a  $\kappa$ B-dependent reporter gene (such as luciferase), or by measuring translocation of NF- $\kappa$ B to the cell nucleus (using methods that are known in the art, e.g., the use of anti-RelB65 antibody to detect nuclear NF- $\kappa$ B). Such screening methods may further comprise detecting whether said test compound binds to  $\lambda$ I $\kappa$ B (or BLIP, or NF- $\kappa$ B), e.g., by preparing cell extracts and immunoprecipitating the protein of interest, and immunoblotting using an antibody directed to the test compound.

**[0066]** The ability of compounds to modulate BLIP autophosphorylation activity can also be used as a method of screening compounds for NF- $\kappa$ B activation or inhibitory effects. In general terms, such screening methods involves administering a test compound to a cell and detecting BLIP autophosphorylation, compared to a control cell in which the test compound is absent.

**[0067]** The present invention also includes within its scope those compounds that are identified as possessing useful NF- $\kappa$ B modulation activity, by the screening methods referred to above.

**[0068]** Another aspect of the present invention is the use of compounds that inhibit or enhance NF- $\kappa$ B activation (including compounds identified by the screening techniques referred to above), in the treatment or prophylaxis of disorders which are responsive to modulation of BLIP activity or modulation of NF- $\kappa$ B activity, in a mammalian (preferably human) patient. The present invention thus provides a method of inhibiting (or enhancing) NF- $\kappa$ B activation in a cell, by providing to the cell a compound that inhibits (or enhances) BLIP-dependent NF- $\kappa$ B activation. An example of such an inhibitory compound is a kinase dead BLIP mutant (e.g., a protein of SEQ ID NO:5), or an antisense nucleotide as described above, where the inhibitory compound is given in an amount effective to decrease endogenous BLIP-dependent NF- $\kappa$ B activation in the cell (compared to NF- $\kappa$ B activation that would occur in the cell in the absence of the inhibitory compound). Compounds that enhance NF- $\kappa$ B activation are provided in an amount effective to increase endogenous BLIP-dependent NF- $\kappa$ B activation in the cell (compared to NF- $\kappa$ B activation that would occur in the cell in the absence of the compound). The providing step may be carried out by any suitable means, such as by delivering the inhibitory compound into the cell, or by delivering into the cell a nucleic acid encoding and expressing the inhibitory compound.

**[0069]** Compounds that inhibit the BLIP-related activation of NF- $\kappa$ B will have therapeutic benefit in pathologies where such activation is upregulated. In many cells of the immune system NF- $\kappa$ B is activated in response to various pathogenic signals, and induces transcription of a variety of genes encoding immunologically relevant proteins. Baeuerle and Henkel, *Annu Rev. Immunol.* 12:141 (1994). Interference with the activation or activity of NF- $\kappa$ B may suppress immunological reactions, including that seen in toxic/septic shock, graft-vs-host reactions, acute inflammatory reactions and radiation damage. In cancerous or neoplastic cells, both chemotherapy and radiation therapy can activate NF- $\kappa$ B, which in turn reduces the apoptotic effects of the therapy. Inhibition of NF- $\kappa$ B activation in tumors has been reported to enhance the apoptotic potential of cancer treatments. Wang et al., *Nature Medicine* 5:412 (1999).

**[0070]** Accordingly, BLIP-mediated NF- $\kappa$ B activation is implicated in immunological and neoplastic disease. Therefore, inhibition of BLIP-mediated NF- $\kappa$ B activation will provide a positive therapeutic benefit where immune suppression is desired, or where anti-neoplastic effects are desired. In particular, such inhibitory compounds will have utility for treatment and/or prophylaxis of disorders such as toxic/septic shock, graft-vs-host reactions, acute inflammatory reactions and radiation damage. Such compounds will further have use as anti-neoplastic agents in the treatment of tumors or cancers, and as adjuvants in the chemotherapy or radiation therapy of neoplastic growths. For example, adenoviral delivery to cancer cells of a kinase-dead mutant of BLIP (or another inhibitor of BLIP-dependent NF- $\kappa$ B activation), will be useful as an adjuvant in the chemotherapy of cancers, to increase the apoptotic effects of the chemotherapies.

**[0071]** Compounds that increase or enhance the BLIP-related activation of NF- $\kappa$ B will have therapeutic benefit in pathologies where such activation is reduced, or where it is desired to upregulate NF- $\kappa$ B activation. Such compounds are useful as immunostimulators and as inhibitors of cell death.

**[0072]** NF- $\kappa$ B modulating compounds, including those identified according to the screening methods outlined above, may be formulated with standard pharmaceutically acceptable carriers and/or excipients as is routine in the pharmaceutical art, and as fully described in Remington's Pharmaceutical Sciences, Mack Publishing Company, Eastern Pennsylvania, 17th Ed, 1985, the disclosure of which is included herein in its entirety by way of reference. The compounds may be administered via enteral or parenteral routes such as via oral, buccal, anal, pulmonary, intravenous, intraarterial, intramuscular, intraperitoneal, topical or other appropriate administration routes.

[0073] The present invention will now be further described, by way of example, in the appended experimental section.

## Example 1

### Materials and Methods

#### Reagents and Cell Cultures:

[0074] Recombinant human TNF $\alpha$  was purchased from Promega; monoclonal 12CA5 anti-Hemagglutinin (anti-HA) antibody from Boehringer; monoclonal antibody against  $\lambda$ PKC from Transduction Laboratories; goat polyclonal anti-p65 (NF- $\kappa$ B) antibody from Santa Cruz Biotechnologies, Inc. The rabbit affinity-purified anti-LIP has been described (Díaz-Meco et al., 1996). Human HEK293 and HeLa cells were obtained from the American Type Culture Collection (ATCC). Cultures of HEK293 cells were maintained in high glucose Dulbecco's modified Eagle's medium containing 10% fetal calf serum, penicillin G (100  $\mu$ g/ml), and streptomycin (100  $\mu$ g/ml) (Flow). HeLa cells were maintained in minimum essential medium Eagle supplemented with 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate and 10% fetal calf serum. Subconfluent cells were transfected by the calcium phosphate method (Clontech Laboratories, Inc.).

#### Plasmids:

[0075] To clone the cDNA for BLIP coding sequence, a human brain Marathon Ready cDNA (Clontech Laboratories, Inc.) was used following the manufacturer's procedures. This cDNA was subcloned into the NotI site of the pCDNA3-HA vector to make the pCDNA3-HA-BLIP construct. pCDNA3-HA-BLIP<sup>MUT</sup> (D1066A; N1071K) was obtained by site directed mutagenesis (Quick Change, Stratagene). pCDNA3-HA- $\lambda$ iPKC and pCDNA3-HA- $\lambda$ iPKC<sup>MUT</sup> have previously been described (Díaz-Meco et al., 1996).

#### Immunoprecipitations:

[0076] For immunoprecipitations of endogenous proteins, HEK293 cells were used. Some cells were stimulated with 30 ng/ml of TNF- $\alpha$ . Cells were then harvested and lysed in buffer PD [40 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% Nonidet P-40, 6 mM EDTA, 6 mM EGTA, 10 mM  $\beta$ -glycerophosphate, 10 mM NaF, 10 mM PNPP, 300  $\mu$ M Na<sub>3</sub>VO<sub>4</sub>, 1mM benzamidine, 2 M PMSF, aprotinin 10  $\mu$ g/ml, leupeptin 1 $\mu$ g/ml, pepstatin 1  $\mu$ g/ml, 1mM DTT], and 1mg of whole-cell lysate was diluted in PD buffer and incubated with 10  $\mu$ g of the monoclonal anti- $\lambda$ iPKC antibody. This reaction mixture was incubated on ice for 2 hours, and then 25  $\mu$ l of protein A or G beads was added and the mixture was left to incubate with gentle rotation for an additional 1 hour at 4°C. The immunoprecipitates were then washed three times with PD buffer. Samples were fractionated on 8% SDS-PAGE, transferred to Nitrocellulose ECL membrane (Amersham) and subjected to Western blot analysis with anti-LIP. Proteins were detected with the ECL reagent (Amersham).

#### Luciferase reporter assays:

[0077] Subconfluent cultures of HEK293 cells were transfected with different amounts of plasmid pCDNA3-HA-BLIP or pCDNA3-HA-BLIP<sup>MUT</sup> together with the  $\kappa$ B-Luc reporter plasmid either with or without expression vectors for wild type and dominant negative  $\lambda$ iPKC (pCDNA3-HA- $\lambda$ iPKC and pCDNA3-HA- $\lambda$ iPKC<sup>MUT</sup>). After 24 hours, cells were stimulated with TNF- $\alpha$  for 4 hours. Extracts were prepared and luciferase activity determined as described (Díaz-Meco et al., 1996).

#### Immunofluorescence:

[0078] HeLa cells were grown on glass coverslips in growth media. Subconfluent cells were transfected with 5  $\mu$ g of HA-BLIP<sup>MUT</sup>. Twenty-hours post-transfection, cells were stimulated with TNF- $\alpha$  (30 ng/ml) for 20 min. Cells were rapidly washed twice in ice-cold PBS, and fixed in 4% formaldehyde for 15 minutes at room temperature. Cells were washed four times with PBS and permeabilized with 0.1 % Triton X-100. Free aldehyde groups were quenched with 50 mM NH<sub>4</sub>Cl. Endogenous peroxidase activity was quenched by treatment with 1% H<sub>2</sub>O<sub>2</sub> in PBS for 15 minutes. The fixed cells were blocked in blocking solution. Cells were incubated with the different antibodies for 1 hour at 37°C. Transfected HA-tagged protein was visualized with the monoclonal 12CA5 anti-HA (Boehringer Mannheim), and an FITC-conjugated anti-mouse (Cappel), and the endogenous p65 with a goat polyclonal antibody (Santa Cruz Biotech), and the tetramethylrhodamine tyramide TSA-Direct amplification system (NEN Life Science Products). Glass coverslips were mounted on Mowiol and were examined with an MRC 1024 Bio-Rad confocal system (Bio-Rad Richmond, CA) mounted

on a Zeiss Axiovert 135 microscope (Zeiss, Oberkochen, Germany).

## Example 2

### 5 Detection of BLIP

[0079] In the course of a series of immunoblot analysis to determine the expression of LIP in different murine organs and tissues, the present inventors observed that LIP was expressed in testicle and heart tissues but not in other tissues investigated (liver, ovary, spleen, thymus, brain, lung and kidney). However, a band that reacted with the anti-LIP antibody and having a molecular weight higher than 200 kDa was unexpectedly detected (results not shown).

[0080] The expression of LIP and the newly identified band was then determined in two different human cell lines routinely used in the laboratory (HEK293 cells and HeLa cells). It was observed that both bands were detectable in both cell lines although the proportion varied, with the high molecular weight protein more abundant in HEK293 than in HeLa cells (results not shown).

## 15 Example 3

### Binding of BLIP to $\lambda$ iPKC

[0081] The present inventors assessed whether BLIP could bind to  $\lambda$ iPKC in vivo. Using HEK293 cells, the cells were either untreated (control) or stimulated with TNF $\alpha$  for various times, after which cell extracts were prepared, immunoprecipitated with a selective anti- $\lambda$ iPKC antibody, and analyzed by immunoblotting with the anti-LIP antibody (results not shown). Under resting conditions there was little or no association of BLIP (or LIP; also not shown) to  $\lambda$ iPKC. However, upon TNF $\alpha$  stimulation there was a rapid and sustained interaction of BLIP with  $\lambda$ iPKC. This indicates that BLIP displays a stimulus-induced association with  $\lambda$ iPKC.

## Example 4

### LIP Inhibits NF- $\kappa$ B Dependent Transcription

[0082] To determine the effect of over-expressing LIP (SEQ ID NO:6) on NF- $\kappa$ B activation, HEK293 cells were transfected with a  $\kappa$ B-dependent reporter gene (luciferase) along with increasing concentrations of an expression plasmid for LIP (0.2, 0.5, 1.0 and 2.0  $\mu$ g/60mm diameter well). Twenty-hour post-transfection cells were either untreated or stimulated with 30 ng/ml TNF $\alpha$ . The results (mean  $\pm$  SD of three independent experiments with incubation in duplicate; Figure 1) demonstrate that the expression of LIP decreases TNF $\alpha$ -induced  $\kappa$ B-dependent reporter activity.

## Example 5

### Cloning of BLIP

[0083] The present inventors next cloned and molecularly characterized human BLIP. The BLIP clone (SEQ ID NO: 3) expanded the LIP sequence previously reported by Diaz-Meco et al. (1996). BLIP was found to be a protein of 2392 amino acids (Fig. 2A, SEQ ID NO:4) with a predicted molecular weight of 269 kDa. The region encompassing amino acids 881-1209 of BLIP displays a high degree of homology with the PI 3-kinase domains of FRAP and ATM (Figs. 3A and 3B). The alignment of the PI 3-kinase-like domains of BLIP, FRAP and ATM indicates that BLIP has kinase activity, as the DXXXXN and DFG motifs that constitute critical residues of the catalytic site conserved in all ATM family members with kinase activity, are also conserved in BLIP. In contrast to FRAP and ATM, however, the kinase region of BLIP is not located at the most C-terminal part of the molecule but roughly in the middle (Fig. 2B). The C-terminus portion of BLIP is the sequence corresponding to LIP. Therefore, LIP constitutes a domain of BLIP (Fig. 2B), revealing a domain organization that differs from other PI 3-kinases. Upstream of the PI 3-kinase domain of FRAP there are a series of protein-protein interaction modules called the HEAT domains (Dennis et al., 1999). Such domains (or other regions through which one could predict interactions with other proteins) are absent in the BLIP molecule, with the exception of the LIP region whereby  $\lambda$ iPKC interacts (Dennis et al., 1999). Another feature of FRAP that is absent in LIP is the FRB domain; this domain is the region responsible for the interaction of FRAP with the FKB/rapamycin complex (Dennis et al., 1999). This suggests that BLIP is rapamycin-insensitive.

**Example 6****Kinase Domain of BLIP**

**[0084]** To investigate whether the FRAP/ATM-like kinase domain of BLIP plays a role in NF- $\kappa$ B activation, HEK293 cells were transfected with a  $\kappa$ B-dependent reporter gene along with increasing concentrations (0.2, 0.5, 1.0, 2.0  $\mu$ g/60mm diameter well) of expression plasmids for BLIP (either wild-type (SEQ ID NO:4) or a kinase-inactive mutant (SEQ ID NO:5)). As a positive control, cells were also transfected with a RIP (Receptor Interacting Protein) expression vector (RIP is a potent activator of NF- $\kappa$ B). The results (**Figure 4A**) indicate that the expression of wild-type BLIP (but not mutant BLIP) is sufficient to activate the  $\kappa$ B-dependent reporter activity to an extent comparable to that of RIP.

**Example 7** **$\lambda$ I $\kappa$ BPKC and BLIP-related NF- $\kappa$ B activation**

**[0085]** To determine whether the NF- $\kappa$ B activation effect of BLIP is dependent on  $\lambda$ I $\kappa$ BPKC, HEK293 cells were transfected with a  $\kappa$ B-dependent luciferase reporter plasmid and one of the following: a negative control vector, an expression plasmid for wild type  $\lambda$ I $\kappa$ BPKC, or an expression plasmid for kinase-inactive  $\lambda$ I $\kappa$ BPKC. Luciferase activity in cells without BLIP (control) and with increasing concentrations (0.5, 1.0, 2.0  $\mu$ g/60mm diameter) of wild-type BLIP (BLIP) was assessed after 24 hours. Results are shown in **Figure 4B**. The co-expression of wild-type  $\lambda$ I $\kappa$ BPKC cooperates with BLIP to activate NF- $\kappa$ B, whereas the expression of the kinase-inactive  $\lambda$ I $\kappa$ BPKC mutant abrogates this effect of BLIP.

**Example 8****Translocation of RelA to the Cell Nucleus**

**[0086]** To further demonstrate that BLIP controls the actual translocation of RelA to the nucleus, HeLa cells were transfected with the HA-tagged BLIP mutant expression vector (BLIP<sup>MUT</sup>); as control cells, non-transfected HeLa cells were used. Twenty-four hours post-transfection the cells were either stimulated with TNF $\alpha$  for 20 minutes or untreated (control). The cells were then analyzed by double immunofluorescence using an anti p65 (RelA) antibody and anti HA-antibody to detect cells that were expressing the construct (data not shown). Analysis of the cells indicates that more than 80% of cells transfected with wildtype BLIP mutant had TNF $\alpha$ -induced translocation of p65, compared to about 20% of cells transfected with BLIP mutant (**Figure 5**).

**Example 9****Screening Methods**

**[0087]** Using an appropriate mammalian cell line (such as HEK293 or HeLa cells), a test compound is administered to the test cells; no test compound is administered to control cells. The cells are then stimulated with TNF $\alpha$  for various times, after which cell extracts are prepared, immunoprecipitated with a selective anti- $\lambda$ I $\kappa$ BPKC antibody, and analyzed by immunoblotting with the anti-BLIP antibody. An increase in the association of BLIP with  $\lambda$ I $\kappa$ BPKC in the test cells (compared to the association found in control cells) indicates that the test compound enhances BLIP-dependent  $\lambda$ I $\kappa$ BPKC activation (and thus NF- $\kappa$ B activation); a decrease indicates the test compound inhibits BLIP-dependent  $\lambda$ I $\kappa$ BPKC activation (and thus NF- $\kappa$ B activation).

**[0088]** Alternatively, the cells may be transfected with a  $\kappa$ B-dependent reporter gene, such as luciferase, and the activity of the reporter gene assessed as a measure of NF- $\kappa$ B activation in the presence of a test compound, compared to control cells.

**References****[0089]**

1. Sambrook, J., Fritsch, E.F. and Maniatis, T. Molecular Cloning: a Laboratory Manual. 2<sup>nd</sup> Edition. CSH Laboratory Press. (1989)
2. Akimoto, K., K. Mizuno, S. Osada, S. Hirai, S. Tanuma, K. Suzuki, and S. Ohno. 1994. A new member of the third class in the Protein Kinase C Family, PKC, expressed dominantly in an undifferentiated mouse embryonal

carcinoma cell line and also in many tissues and cells. J. Biol. Chem. 269: 12677-12683.

3. Baeuerle, P.A., and T. Henkel. 1994. Function and activation of NF- $\kappa$ B in the immune system. Annu. Rev. Immunol. 12: 141-179.

4. Baldwin, A.S. 1996. The NF- $\kappa$ B and I $\kappa$ B proteins: new discoveries and insights. Annu. Rev. Immunol. 14: 649-683.

5. Berghe, W.V., S. Plaisance, E. Boone, K. De Bosscher, M.L. Schmitz, W. Fiers, and G. Haegeman. 1998. p38 and the extracellular signal-regulated kinase mitogen-activated protein kinase pathways are required for nuclear factor- $\kappa$ B p65 transactivation mediated by tumor necrosis factor. J. Biol. Chem. 273: 3285-3290.

6. Berra, E., M.T. Diaz-Meco, J. Lozano, S. Frutos, M.M. Municio, P. Sanchez, L. Sanz, and J. Moscat. 1995. Evidence for a role of MEK and MAPK during signal transduction by protein kinase C  $\zeta$ . EMBO J. 14: 6157-6163.

7. Bjorkoy, G., A. Overvatn, M.T. Díaz-Meco, J. Moscat, and T. Johansen. 1995. Evidence for a bifurcation of the mitogenic signaling pathway activated by ras and phosphatidylcholine-hydrolyzing. J. Biol. Chem. 270: 21299-21306.

8. Dennis, P.B., S. Fumagalli, and G. Thomas. 1999. Target of rapamycin (TOR): balancing the opposite forces of protein synthesis and degradation. Curr. Opin. Gen. & Devel. 9: 49-54.

9. Díaz-Meco, M.T., E. Berra, M.M. Municio, L. Sanz, J. Lozano, V. Díaz-Golpe, M.T. Lain de Lera, J. Alcamí, C. V. Payá, F. Arenzana-Seisdedos, J.L. Virelizier, and J. Moscat. 1993. A dominant negative protein kinase C  $\zeta$  subspecies blocks NF- $\kappa$ B activation. Mol. Cell. Biol. 13: 4770-4775.

10. Díaz-Meco, M.T., J. Lozano, M.M. Municio, E. Berra, S. Frutos, L. Sanz, and J. Moscat. 1994.  $\zeta$ PKC Induces Phosphorylation and Inactivation of I $\kappa$ B- $\alpha$  In Vitro. EMBO J. 13: 2842-2848.

11. Diaz-Meco, M.T., J. Lozano, M.M. Municio, E. Berra, S. Frutos, L. Sanz, and J. Moscat. 1994. Evidence for the in vitro and in vivo interaction of Ras with protein kinase C  $\zeta$ . J. Biol. Chem. 269: 31706-31710.

12. Diaz-Meco, M.T., M.M. Municio, P. Sanchez, J. Lozano, and J. Moscat. 1996. Lambda-interacting protein, a novel protein that specifically interacts with the zinc finger domain of the atypical protein kinase C isotype  $\lambda/\mu$  and stimulates its kinase activity *in vitro* and *in vivo*. Mol. Cell. Biol. 16:105-114.

13. DiDonato, J.A., M. Hayakawa, D.M. Rothwarf, E. Zandi, and M. Karin. 1997. A cytokine-responsive I $\kappa$ B kinase that activates the transcription factor NF- $\kappa$ B. Nature 388: 548-554.

14. Dominguez, I., L. Sanz, F. Arenzana-Seisdedos, M.T. Díaz-Meco, J.L. Virelizier, and J. Moscat. 1993. Inhibition of protein kinase  $\zeta$  subspecies blocks the activation of a NF- $\kappa$ B-like activity in *Xenopus laevis* oocytes. Mol. Cell. Biol. 13:1290-1295.

15. Folgueira, L., J.A. McElhinny, G.D. Bren, W.S. MacMorrán, M.T. Díaz-Meco, J. Moscat, and C.V. Payá. 1996. Protein kinase C- $\zeta$  mediates NF- $\kappa$ B activation in human immunodeficiency virus-infected monocytes. J. Virol. 70: 223-231.

16. Jung, M., Y. Zhang, S. Lee and A. Dritschilo. 1995. Correction of radiation sensitivity in ataxia telangiectasia cells by a truncated I kappa B-alpha. Science 268:1619-21.

17. Lallena, M. J., M.T. Diaz-Meco, G. Bren, C.V. Payá, and J. Moscat. 1999. Activation of I $\kappa$ B Kinase  $\beta$  by Protein Kinase C Isoforms. Mol. Cell. Biol. 19: 2180-2188.

18. Lenardo, M., and D. Baltimore. 1989. NF- $\kappa$ B: a pleiotropic mediator of inducible and tissue-specific gene control. Cell 58: 227-229.

19. Liao, D.F., B. Monia, N. Dean, and B.C. Berk. 1997. Protein kinase C- $\zeta$  mediates angiotensin II activation of ERK1/2 in vascular smooth muscle cells. J. Biol. Chem. 272: 6146-6150.

20. Lozano, J., E. Berra, M.M. Municio, M.T. Diaz-Meco, I. Dominguez, L. Sanz, and J. Moscat. 1994. Protein kinase C  $\zeta$  isoform is critical for  $\kappa$ B-dependent promoter activation by sphingomyelinase. J. Biol. Chem. 269:19200-19202.
21. Malinin, N.L., M.P. Boldin, A.V. Kovalenko, and D. Wallach. 1997. MAP3K-related kinase involved in NF- $\kappa$ B induction by TNF, CD95 and IL-1. Nature 385: 540-544.
22. Mercurio, F., H. Zhu, B.W. Murray, A. Shevchenko, B.L. Bennett, J.W. Li, D.B. Young, M. Barbosa, M. Mann, A. Manning, and A. Rao. 1997. IKK-1 and IKK-2: cytokine-activated I $\kappa$ B kinases essential for NF- $\kappa$ B activation. Science 278: 860-866.
23. Müller, G., M. Ayoub, P. Storz, J. Rennecke, D. Fabbro, and K. Pfizenmaier. 1995. PKC $\zeta$  is a molecular switch in signal transduction of TNF $\alpha$ , bifunctionally regulated by ceramide and arachidonic acid. EMBO J. 14: 1961-1969.
24. Murray, N.R. and A.P. Fields. 1997. Atypical protein kinase C  $\iota$  protects human leukemia cells against drug-induced apoptosis. J. Biol. Chem. 272: 27521-27524.
25. Nakanishi, H., K.A. Brewer, and J.H. Exton. (1993). Activation of the isozyme of protein kinase C by phosphatidylinositol 3,4,5-trisphosphate J. Biol. Chem. 268: 13-16.
26. Réginer, C.H., H.Y. Song, X. Gao, D.V. Goeddel, Z. Cao, and M. Rothe. 1997. Identification and characterization of an I $\kappa$ B kinase. Cell 90: 373-383.
27. Romanelli, A. K. A. Martin, A. Toker, and J. Blenis. 1999. P70 S6 kinase is regulated by protein kinase c zeta and participates in a phosphoinositide 3-kinase-regulated signalling complex. Mol. Cell Biol. 19:2921-8.
28. Rzymkiewicz, D.M., T. Tetsuka, D. Daphna-Iken, S. Srivastava, and A.R. Morrison. 1996. Interleukin-1 $\beta$  activates protein kinase C  $\zeta$  in renal mesangial cells. J. Biol. Chem. 271: 17241-17246.
29. Schönwasser, D.C., R.M. Marais, C.J. Marshall, and P.J. Parker. 1998. Activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway by conventional, novel, and atypical protein kinase C isotypes. Mol. Cell. Biol. 18: 790-798.
30. Sontag, E., J.M. Sontag, and A. Garcia. 1997. Protein phosphatase 2A is a critical regulator of protein kinase C  $\zeta$  signaling targeted by SV40 small t to promote cell growth and NF- $\kappa$ B activation. EMBO J. 16: 5662-5671.
31. Stancovski, I., and D. Baltimore. 1997. NF- $\kappa$ B activation: The I $\kappa$ B kinase revealed? Cell 91: 299-302.
32. Takeda, H., T. Matozaki, T. Takada, T. Noguchi, T. Yamao, M. Tsuda, F. Ochi, K. Fukunaga, K. Inagaki, and M. Kasuga. 1999. PI 3-kinase gamma and protein kinase C- $\zeta$  mediate RAS-independent activation of MAP kinase by a Gi protein-coupled receptor. EMBO J 18:386-395.
33. Thanos, D., and T. Maniatis. 1995. NF- $\kappa$ B: a lesson in family values. Cell 80: 529-532.
34. Verma, I.M., J.K. Stevenson, E.M. Schwarz, D. VanAntwerp, and S. Miyamoto. 1995. Rel/NF- $\kappa$ B/I $\kappa$ B family: intimate tales of association and dissociation. Genes Dev. 9: 2723-2735.
35. Woronicz, J.D., X. Gao, Z. Cao, M. Rothe and D.V. Goeddel. 1997. I $\kappa$ B kinase- $\beta$ : NF- $\kappa$ B activation and complex formation with I $\kappa$ B kinase- $\alpha$  and NIK. Science 278: 866-869.
36. Zandi, E., D.M. Rothwarf, M. Delhase, M. Hayakawa, and M. Karin. 1997. The I $\kappa$ B kinase complex (IKK) contains two kinase subunits, IKK $\alpha$  and IKK $\beta$ , necessary for I $\kappa$ B phosphorylation and NF- $\kappa$ B activation. Cell 91: 243-252.

## SEQUENCE LISTING

<110> Glaxo Wellcome, S.A.

<120> Protein Kinase C Interacting Protein Involved in NF-KB  
Activation

<130> PI 3718

<140>

<141>

<160> 8

<170> PatentIn Ver. 2.0

<210> 1

<211> 309

<212> PRT

<213> Homo sapiens

<400> 1

Leu Glu Leu Gln Tyr Val Ser Pro Lys Leu Leu Met Cys Arg Asp Leu  
1 5 10 15

Glu Leu Ala Val Pro Gly Thr Tyr Asp Pro Asn Gln Pro Ile Ile Arg  
20 25 30

Ile Gln Ser Ile Ala Pro Ser Leu Gln Val Ile Thr Ser Lys Gln Arg  
35 40 45

Pro Arg Lys Leu Thr Leu Met Gly Ser Asn Gly His Glu Phe Val Phe  
50 55 60

Leu Leu Lys Gly His Glu Asp Leu Arg Gln Asp Glu Arg Val Met Gln  
65 70 75 80

Leu Phe Gly Leu Val Asn Thr Leu Leu Ala Asn Asp Pro Thr Ser Leu  
85 90 95

Arg Lys Asn Leu Ser Ile Gln Arg Tyr Ala Val Ile Pro Leu Ser Thr  
100 105 110

Asn Ser Gly Leu Ile Gly Trp Val Pro His Cys Asp Thr Leu His Ala  
115 120 125

Leu Ile Arg Asp Tyr Arg Glu Lys Lys Lys Ile Leu Leu Asn Ile Glu  
130 135 140

EP 1 231 264 A1

His Arg Ile Met Leu Arg Met Ala Pro Asp Tyr Asp His Leu Thr Leu  
 145 150 155 160  
 5 Met Gln Lys Val Glu Val Phe Glu His Ala Val Asn Asn Thr Ala Gly  
 165 170 175  
 Asp Asp Leu Ala Lys Leu Leu Trp Leu Lys Ser Pro Ser Ser Glu Val  
 10 180 185 190  
 Trp Phe Asp Arg Arg Thr Asn Tyr Thr Arg Ser Leu Ala Val Met Ser  
 195 200 205  
 15 Met Val Gly Tyr Ile Leu Gly Leu Gly Asp Arg His Pro Ser Asn Leu  
 210 215 220  
 Met Leu Asp Arg Leu Ser Gly Lys Ile Leu His Ile Asp Phe Gly Asp  
 20 225 230 235 240  
 Cys Phe Glu Val Ala Met Thr Arg Glu Lys Phe Pro Glu Lys Ile Pro  
 245 250 255  
 Phe Arg Leu Thr Arg Met Leu Thr Asn Ala Met Glu Val Thr Gly Leu  
 25 260 265 270  
 Asp Gly Asn Tyr Arg Ile Thr Cys His Thr Val Met Glu Val Leu Arg  
 275 280 285  
 30 Glu His Lys Asp Ser Val Met Ala Val Leu Glu Ala Phe Val Tyr Asp  
 290 295 300  
 Pro Leu Leu Asn Trp  
 305  
 35  
 <210> 2  
 <211> 120  
 <212> PRT  
 40 <213> Homo sapiens  
 <400> 2  
 Pro Ala Ile Trp Phe Glu Lys Arg Leu Ala Tyr Thr Arg Ser Val Ala  
 1 5 10 15  
 45 Thr Ser Ser Ile Val Gly Tyr Ile Leu Gly Leu Gly Asp Arg His Val  
 20 25 30  
 Gln Asn Ile Leu Ile Asn Glu Gln Ser Ala Glu Leu Val His Ile Asp  
 35 40 45  
 50  
 55



EP 1 231 264 A1

Leu Gly Val Ala Phe Glu Gln Gly Lys Ile Leu Pro Thr Pro Glu Thr  
 50 55 60  
 5 Val Pro Phe Arg Leu Thr Arg Asp Ile Val Asp Gly Met Gly Ile Thr  
 65 70 75 80  
 Gly Val Glu Gly Val Phe Arg Arg Cys Cys Glu Lys Thr Met Glu Val  
 85 90 95  
 10 Met Arg Asn Ser Gln Glu Thr Leu Leu Thr Ile Val Glu Val Leu Leu  
 100 105 110  
 Tyr Asp Pro Leu Phe Asp Trp Thr  
 115 120  
 15  
 <210> 3  
 <211> 9138  
 20 <212> DNA  
 <213> Homo sapiens  
 <220>  
 <221> CDS  
 25 <222> (152)..(7327)  
 <400> 3  
 agaaaaaata gagtttcact ctgttgccca ggctggagtg cagtgacgca atctcggtc 60  
 30 actgcagcct ctgccctctg agttcaagtg attctcctgc ctcagcctcc tgagtagctg 120  
 ggattacagg tgtctgccac cgtgccaga c atg aaa aaa ctg ctt cct aac 172  
 Met Lys Lys Leu Leu Pro Asn  
 1 5  
 35 atg tta agt ccg gat ccg agg gaa ctt cag aaa tcc att gaa gtt caa 220  
 Met Leu Ser Pro Asp Pro Arg Glu Leu Gln Lys Ser Ile Glu Val Gln  
 10 15 20  
 40 ttg tta aga agt tct gtt tgt ttg gca act gct tta aac ccg ata gaa 268  
 Leu Leu Arg Ser Ser Val Cys Leu Ala Thr Ala Leu Asn Pro Ile Glu  
 25 30 35  
 45 caa gat cag aag tgg cag tct ata act gaa aat gtg gta aag tac ttg 316  
 Gln Asp Gln Lys Trp Gln Ser Ile Thr Glu Asn Val Val Lys Tyr Leu  
 40 45 50 55  
 50 aag caa aca tcc cgc atc gct att gga cct ctg aga ctt tct act tta 364  
 Lys Gln Thr Ser Arg Ile Ala Ile Gly Pro Leu Arg Leu Ser Thr Leu  
 60 65 70

55

EP 1 231 264 A1

5  
aca gtt tca cag tct ttg cca gtt cta agt acc ttg cag ctg tat tgc 412  
Thr Val Ser Gln Ser Leu Pro Val Leu Ser Thr Leu Gln Leu Tyr Cys  
75 80 85

10  
tca tct gct ttg gag aac aca gtt tct aac aga ctt tca aca gag gac 460  
Ser Ser Ala Leu Glu Asn Thr Val Ser Asn Arg Leu Ser Thr Glu Asp  
90 95 100

15  
tgt ctt att cca ctc ttc agt gaa gct tta cgt tca tgt aaa cag cat 508  
Cys Leu Ile Pro Leu Phe Ser Glu Ala Leu Arg Ser Cys Lys Gln His  
105 110 115

20  
gac gtg agg cca tgg atg cag gca tta agg tat act atg tac cag aat 556  
Asp Val Arg Pro Trp Met Gln Ala Leu Arg Tyr Thr Met Tyr Gln Asn  
120 125 130 135

25  
cag ttg ttg gag aaa att aaa gaa caa aca gtc cca att aga agc cat 604  
Gln Leu Leu Glu Lys Ile Lys Glu Gln Thr Val Pro Ile Arg Ser His  
140 145 150

30  
ctc atg gaa tta ggt cta aca gca gca aaa ttt gct aga aaa cga ggg 652  
Leu Met Glu Leu Gly Leu Thr Ala Ala Lys Phe Ala Arg Lys Arg Gly  
155 160 165

35  
aat gtg tcc ctt gca aca aga ctg ctg gca cag tgc agt gaa gtt cag 700  
Asn Val Ser Leu Ala Thr Arg Leu Leu Ala Gln Cys Ser Glu Val Gln  
170 175 180

40  
ctg gga aag acc acc act gca cag gat tta gtc caa cat ttt aaa aaa 748  
Leu Gly Lys Thr Thr Thr Ala Gln Asp Leu Val Gln His Phe Lys Lys  
185 190 195

45  
cta tca acc caa ggt caa gtg gat gaa aaa tgg ggg ccc gaa ctt gat 796  
Leu Ser Thr Gln Gly Gln Val Asp Glu Lys Trp Gly Pro Glu Leu Asp  
200 205 210 215

50  
att gaa aaa acc aaa ttg ctt tat aca gca ggc cag tca aca cat gca 844  
Ile Glu Lys Thr Lys Leu Leu Tyr Thr Ala Gly Gln Ser Thr His Ala  
220 225 230

55  
atg gaa atg ttg agt tct tgt gcc ata tct ttc tgc aag tct gtg aaa 892  
Met Glu Met Leu Ser Ser Cys Ala Ile Ser Phe Cys Lys Ser Val Lys  
235 240 245

gct gaa tat gca gtt gct aaa tca att ctg aca ctg gct aaa tgg atc 940  
Ala Glu Tyr Ala Val Ala Lys Ser Ile Leu Thr Leu Ala Lys Trp Ile  
250 255 260

EP 1 231 264 A1

5  
 10  
 15  
 20  
 25  
 30  
 35  
 40  
 45  
 50  
 55

cag gca gaa tgg aaa gag att tca gga cag ctg aaa cag gtt tac aga 988  
 Gln Ala Glu Trp Lys Glu Ile Ser Gly Gln Leu Lys Gln Val Tyr Arg  
 265 270 275

gct cag cac caa cag aac ttc aca ggt ctt tct act ttg tct aaa aac 1036  
 Ala Gln His Gln Gln Asn Phe Thr Gly Leu Ser Thr Leu Ser Lys Asn  
 280 285 290 295

ata ctc act cta ata gaa ctg cca tct gtt aat acg atg gaa gaa gag 1084  
 Ile Leu Thr Leu Ile Glu Leu Pro Ser Val Asn Thr Met Glu Glu Glu  
 300 305 310

tat cct cgg atc gag agt gaa tct aca gtg cat att gga gtt gga gaa 1132  
 Tyr Pro Arg Ile Glu Ser Glu Ser Thr Val His Ile Gly Val Gly Glu  
 315 320 325

cct gac ttc att ttg gga cag ttg tat cac ctg tct tca gta cag gca 1180  
 Pro Asp Phe Ile Leu Gly Gln Leu Tyr His Leu Ser Ser Val Gln Ala  
 330 335 340

cct gaa gta gcc aaa tct tgg gca gcg ttg gcc agc tgg gct tat agg 1228  
 Pro Glu Val Ala Lys Ser Trp Ala Ala Leu Ala Ser Trp Ala Tyr Arg  
 345 350 355

tgg ggc aga aag gtg gtt gac aat gcc agt cag gga gaa ggt gtt cgt 1276  
 Trp Gly Arg Lys Val Val Asp Asn Ala Ser Gln Gly Glu Gly Val Arg  
 360 365 370 375

ctg ctg cct aga gaa aaa tct gaa gtt cag aat cta ctt cca gac act 1324  
 Leu Leu Pro Arg Glu Lys Ser Glu Val Gln Asn Leu Leu Pro Asp Thr  
 380 385 390

ata act gag gaa gag aaa gag aga ata tat ggt att ctt gga cag gct 1372  
 Ile Thr Glu Glu Glu Lys Glu Arg Ile Tyr Gly Ile Leu Gly Gln Ala  
 395 400 405

gtg tgt cgg ccg gcg ggg att cag gat gaa gat ata aca ctt cag ata 1420  
 Val Cys Arg Pro Ala Gly Ile Gln Asp Glu Asp Ile Thr Leu Gln Ile  
 410 415 420

act gag agt gaa gac aac gaa gaa gat gac atg gtt gat gtt atc tgg 1468  
 Thr Glu Ser Glu Asp Asn Glu Glu Asp Asp Met Val Asp Val Ile Trp  
 425 430 435

cgt cag ttg ata tca agc tgc cca tgg ctt tca gaa ctt gat gaa agt 1516  
 Arg Gln Leu Ile Ser Ser Cys Pro Trp Leu Ser Glu Leu Asp Glu Ser  
 440 445 450 455

gca act gaa gga gtt att aaa gtg tgg agg aaa gtt gta gat aga ata 1564

EP 1 231 264 A1

	Ala Thr Glu Gly Val Ile Lys Val Trp Arg Lys Val Val Asp Arg Ile	
	460 465 470	
5	ttc agc ctg tac aaa ctc tct tgc agt gca tac ttt act ttc ctt aaa Phe Ser Leu Tyr Lys Leu Ser Cys Ser Ala Tyr Phe Thr Phe Leu Lys	1612
	475 480 485	
10	ctc aac gct ggt caa att cct tta gat gag gat gac cct agg ctg cat Leu Asn Ala Gly Gln Ile Pro Leu Asp Glu Asp Asp Pro Arg Leu His	1660
	490 495 500	
15	tta agt cac aga gtg gaa cag agc act gat gac atg att gtg atg gcc Leu Ser His Arg Val Glu Gln Ser Thr Asp Asp Met Ile Val Met Ala	1708
	505 510 515	
20	aca ttg cgc ctg ctg cgg ttg ctc gtg aag cac gct ggt gag ctt cgg Thr Leu Arg Leu Leu Arg Leu Leu Val Lys His Ala Gly Glu Leu Arg	1756
	520 525 530 535	
	cag tat ctg gag cac ggc ttg gag aca aca ccc act gca cca tgg aga Gln Tyr Leu Glu His Gly Leu Glu Thr Thr Pro Thr Ala Pro Trp Arg	1804
	540 545 550	
25	gga att att ccg caa ctt ttc tca cgc tta aac cac cct gaa gtg tat Gly Ile Ile Pro Gln Leu Phe Ser Arg Leu Asn His Pro Glu Val Tyr	1852
	555 560 565	
30	gtg cgc caa agt att tgt aac ctt ctc tgc cgt gtg gct caa gat tcc Val Arg Gln Ser Ile Cys Asn Leu Leu Cys Arg Val Ala Gln Asp Ser	1900
	570 575 580	
35	cca cat ctc ata ttg tat cct gca ata gtg ggt acc ata tcg ctt agt Pro His Leu Ile Leu Tyr Pro Ala Ile Val Gly Thr Ile Ser Leu Ser	1948
	585 590 595	
40	agt gaa tcc cag gct tca gga aat aaa ttt tcc act gca att cca act Ser Glu Ser Gln Ala Ser Gly Asn Lys Phe Ser Thr Ala Ile Pro Thr	1996
	600 605 610 615	
45	tta ctt ggc aat att caa gga gaa gaa ttg ctg gtt tct gaa tgt gag Leu Leu Gly Asn Ile Gln Gly Glu Glu Leu Leu Val Ser Glu Cys Glu	2044
	620 625 630	
	gga gga agt cct cct gca tct cag gat agc aat aag gat gaa cct aaa Gly Gly Ser Pro Pro Ala Ser Gln Asp Ser Asn Lys Asp Glu Pro Lys	2092
	635 640 645	
50	agt gga tta aat gaa gac caa gcc atg atg cag gat tgt tac agc aaa Ser Gly Leu Asn Glu Asp Gln Ala Met Met Gln Asp Cys Tyr Ser Lys	2140

55

EP 1 231 264 A1

	650	655	660	
5	att gta gat aag ctg tcc tct gca aac ccc acc atg gta tta cag gtt Ile Val Asp Lys Leu Ser Ser Ala Asn Pro Thr Met Val Leu Gln Val 665 670 675	2188		
10	cag atg ctc gtg gct gaa ctg cgc agg gtc act gtg ctc tgg gat gag Gln Met Leu Val Ala Glu Leu Arg Arg Val Thr Val Leu Trp Asp Glu 680 685 690 695	2236		
15	ctc tgg ctg gga gtt ttg ctg caa caa cac atg tat gtc ctg aga cga Leu Trp Leu Gly Val Leu Leu Gln Gln His Met Tyr Val Leu Arg Arg 700 705 710	2284		
20	att cag cag ctt gaa gat gag gtg aag aga gtc cag aac aac aac acc Ile Gln Gln Leu Glu Asp Glu Val Lys Arg Val Gln Asn Asn Asn Thr 715 720 725	2332		
25	tta cgc aaa gaa gag aaa att gca atc atg agg gag aag cac aca gct Leu Arg Lys Glu Glu Lys Ile Ala Ile Met Arg Glu Lys His Thr Ala 730 735 740	2380		
30	ttg atg aag ccc atc gta ttt gct ttg gag cat gtg agg agt atc aca Leu Met Lys Pro Ile Val Phe Ala Leu Glu His Val Arg Ser Ile Thr 745 750 755	2428		
35	gcg gct cct gca gaa aca cct cat gaa aaa tgg ttt cag gat aac tat Ala Ala Pro Ala Glu Thr Pro His Glu Lys Trp Phe Gln Asp Asn Tyr 760 765 770 775	2476		
40	ggg gat gcc att gaa aat gcc cta gaa aaa ctg aag act cca ttg aac Gly Asp Ala Ile Glu Asn Ala Leu Glu Lys Leu Lys Thr Pro Leu Asn 780 785 790	2524		
45	cct gca aag cct ggg agc agc tgg att cca ttt aaa gag ata atg cta Pro Ala Lys Pro Gly Ser Ser Trp Ile Pro Phe Lys Glu Ile Met Leu 795 800 805	2572		
50	aat ttg caa cag aga gca cag aaa cgt gca agt tac atc ttg cgt ctt Asn Leu Gln Gln Arg Ala Gln Lys Arg Ala Ser Tyr Ile Leu Arg Leu 810 815 820	2620		
55	gaa gaa atc agt cca tgg ttg gct gcc atg act aac act gaa att gct Glu Glu Ile Ser Pro Trp Leu Ala Ala Met Thr Asn Thr Glu Ile Ala 825 830 835	2668		
60	ctt cct ggg gaa gtc tca gcc aga gac act gtc aca atc cat agt gtg Leu Pro Gly Glu Val Ser Ala Arg Asp Thr Val Thr Ile His Ser Val 840 845 850 855	2716		

EP 1 231 264 A1

5	ggc gga acc atc aca atc tta ccg act aaa acc aag cca aag aaa ctt Gly Gly Thr Ile Thr Ile Leu Pro Thr Lys Thr Lys Pro Lys Lys Leu 860 865 870	2764
10	ctc ttt ctt gga tca gat ggg aag agc tat cct tat ctt ttc aaa gga Leu Phe Leu Gly Ser Asp Gly Lys Ser Tyr Pro Tyr Leu Phe Lys Gly 875 880 885	2812
15	ctg gag gat tta cat ctg gat gag aga ata atg cag ttc cta tct att Leu Glu Asp Leu His Leu Asp Glu Arg Ile Met Gln Phe Leu Ser Ile 890 895 900	2860
20	gtg aat acc atg ttt gct aca att aat cgc caa gaa aca ccc cgg ttc Val Asn Thr Met Phe Ala Thr Ile Asn Arg Gln Glu Thr Pro Arg Phe 905 910 915	2908
25	cat gct cga cac tat tct gta aca cca cta gga aca aga tca gga cta His Ala Arg His Tyr Ser Val Thr Pro Leu Gly Thr Arg Ser Gly Leu 920 925 930 935	2956
30	atc cag tgg gta gat gga gcc aca ccc tta ttt ggt ctt tac aaa cga Ile Gln Trp Val Asp Gly Ala Thr Pro Leu Phe Gly Leu Tyr Lys Arg 940 945 950	3004
35	tgg caa caa cgg gaa gct gcc tta caa gca caa aag gcc caa gat tcc Trp Gln Gln Arg Glu Ala Ala Leu Gln Ala Gln Lys Ala Gln Asp Ser 955 960 965	3052
40	tac caa act cct cag aat cct gga att gta ccc cgt cct agt gaa ctt Tyr Gln Thr Pro Gln Asn Pro Gly Ile Val Pro Arg Pro Ser Glu Leu 970 975 980	3100
45	tat tac agt aaa att ggc cct gct ttg aaa aca gtt ggg ctt agc ctg Tyr Tyr Ser Lys Ile Gly Pro Ala Leu Lys Thr Val Gly Leu Ser Leu 985 990 995	3148
50	gat gtg tcc cgt cgg gat tgg cct ctt cat gta atg aag gca gta ttg Asp Val Ser Arg Arg Asp Trp Pro Leu His Val Met Lys Ala Val Leu 1000 1005 1010 1015	3196
55	gaa gag tta atg gag gcc aca ccc ccg aat ctc ctt gcc aaa gag ctc Glu Glu Leu Met Glu Ala Thr Pro Pro Asn Leu Leu Ala Lys Glu Leu 1020 1025 1030	3244
	tgg tca tct tgc aca aca cct gat gaa tgg tgg aga gtt acg cag tct Trp Ser Ser Cys Thr Thr Pro Asp Glu Trp Trp Arg Val Thr Gln Ser 1035 1040 1045	3292

EP 1 231 264 A1

5	tat gca aga tct act gca gtc atg tct atg gtt gga tac ata att ggc Tyr Ala Arg Ser Thr Ala Val Met Ser Met Val Gly Tyr Ile Ile Gly 1050 1055 1060	3340
10	ctt gga gac aga cat ctg gat aat gtt ctt ata gat atg acg act gga Leu Gly Asp Arg His Leu Asp Asn Val Leu Ile Asp Met Thr Thr Gly 1065 1070 1075	3388
15	gaa gtt gtt cac ata gat tac aat gtt tgc ttt gaa aaa ggt aaa agc Glu Val Val His Ile Asp Tyr Asn Val Cys Phe Glu Lys Gly Lys Ser 1080 1085 1090 1095	3436
20	ctt aga gtt cct gag aaa gta cct ttt cga atg aca caa aac att gaa Leu Arg Val Pro Glu Lys Val Pro Phe Arg Met Thr Gln Asn Ile Glu 1100 1105 1110	3484
25	aca gca ctg ggt gta act gga gta gaa ggt gta ttt agg ctt tca tgt Thr Ala Leu Gly Val Thr Gly Val Glu Gly Val Phe Arg Leu Ser Cys 1115 1120 1125	3532
30	gag cag gtt tta cac att atg cgg cgt ggc aga gag acc ctg ctg acg Glu Gln Val Leu His Ile Met Arg Arg Gly Arg Glu Thr Leu Leu Thr 1130 1135 1140	3580
35	ctg ctg gag gcc ttt gtg tac gac cct ctg gtg gac tgg aca gca gga Leu Leu Glu Ala Phe Val Tyr Asp Pro Leu Val Asp Trp Thr Ala Gly 1145 1150 1155	3628
40	ggc gag gct ggg ttt gct ggt gct gtc tat ggt gga ggt ggc cag cag Gly Glu Ala Gly Phe Ala Gly Ala Val Tyr Gly Gly Gly Gly Gln Gln 1160 1165 1170 1175	3676
45	gcc gag agc aag cag agc aag aga gag atg gag cga gag atc acc cgc Ala Glu Ser Lys Gln Ser Lys Arg Glu Met Glu Arg Glu Ile Thr Arg 1180 1185 1190	3724
50	agc ctg ttt tct tct aga gta gct gag att aag gtg aac tgg ttt aag Ser Leu Phe Ser Ser Arg Val Ala Glu Ile Lys Val Asn Trp Phe Lys 1195 1200 1205	3772
55	aat aga gat gag atg ctg gtt gtg ctt ccc aag ttg gac ggt agc tta Asn Arg Asp Glu Met Leu Val Val Leu Pro Lys Leu Asp Gly Ser Leu 1210 1215 1220	3820
	gat gaa tac cta agc ttg caa gag caa ctg aca gat gtg gaa aaa ctg Asp Glu Tyr Leu Ser Leu Gln Glu Gln Leu Thr Asp Val Glu Lys Leu 1225 1230 1235	3868
	cag ggc aaa cta ctg gag gaa ata gag ttt cta gaa gga gct gaa ggg	3916

EP 1 231 264 A1

	Gln Gly Lys Leu Leu Glu Glu Ile Glu Phe Leu Glu Gly Ala Glu Gly	
	1240 1245 1250 1255	
5	gtg gat cat cct tct cat act ctg caa cac agg tat tct gag cac acc	3964
	Val Asp His Pro Ser His Thr Leu Gln His Arg Tyr Ser Glu His Thr	
	1260 1265 1270	
10	caa cta cag act cag caa aga gct gtt cag gaa gca atc cag gtg aag	4012
	Gln Leu Gln Thr Gln Gln Arg Ala Val Gln Glu Ala Ile Gln Val Lys	
	1275 1280 1285	
15	ctg aat gaa ttt gaa caa tgg ata aca cat tat cag gct gca ttc aat	4060
	Leu Asn Glu Phe Glu Gln Trp Ile Thr His Tyr Gln Ala Ala Phe Asn	
	1290 1295 1300	
20	aat tta gaa gca aca cag ctt gca agc ttg ctt caa gag ata agc aca	4108
	Asn Leu Glu Ala Thr Gln Leu Ala Ser Leu Leu Gln Glu Ile Ser Thr	
	1305 1310 1315	
25	caa atg gac ctt ggt cct cca agt tac gtg cca gca aca gcc ttt ctg	4156
	Gln Met Asp Leu Gly Pro Pro Ser Tyr Val Pro Ala Thr Ala Phe Leu	
	1320 1325 1330 1335	
30	cag aat gct ggt cag gcc cac ttg att agc cag tgc gag cag ctg gag	4204
	Gln Asn Ala Gly Gln Ala His Leu Ile Ser Gln Cys Glu Gln Leu Glu	
	1340 1345 1350	
35	ggg gag gtt ggt gct ctc ctg cag cag agg cgc tcc gtg ctc cgt ggc	4252
	Gly Glu Val Gly Ala Leu Leu Gln Gln Arg Arg Ser Val Leu Arg Gly	
	1355 1360 1365	
40	tgt ctg gag caa ctg cat cac tat gca acc gtg gcc ctg cag tat ccg	4300
	Cys Leu Glu Gln Leu His His Tyr Ala Thr Val Ala Leu Gln Tyr Pro	
	1370 1375 1380	
45	aag gcc ata ttt cag aaa cat cga att gaa cag tgg aag acc tgg atg	4348
	Lys Ala Ile Phe Gln Lys His Arg Ile Glu Gln Trp Lys Thr Trp Met	
	1385 1390 1395	
50	gaa gag ctc atc tgt aac acc aca gta gag cgt tgt caa gag ctc tat	4396
	Glu Glu Leu Ile Cys Asn Thr Thr Val Glu Arg Cys Gln Glu Leu Tyr	
	1400 1405 1410 1415	
55	agg aaa tat gaa atg caa tat gct ccc cag cca ccc cca aca gtg tgt	4444
	Arg Lys Tyr Glu Met Gln Tyr Ala Pro Gln Pro Pro Pro Thr Val Cys	
	1420 1425 1430	
60	cag ttc atc act gcc act gaa atg acc ctg cag cga tac gca gca gac	4492
	Gln Phe Ile Thr Ala Thr Glu Met Thr Leu Gln Arg Tyr Ala Ala Asp	



EP 1 231 264 A1

	1435	1440	1445	
5	atc aac agc aga ctt att aga caa gtg gaa cgc ttg aaa cag gaa gct Ile Asn Ser Arg Leu Ile Arg Gln Val Glu Arg Leu Lys Gln Glu Ala 1450 1455 1460	4540		
10	gtc act gtg cca gtt tgt gaa gat cag ttg aaa gaa att gaa cgt tgc Val Thr Val Pro Val Cys Glu Asp Gln Leu Lys Glu Ile Glu Arg Cys 1465 1470 1475	4588		
15	att aaa gtt ttc ctt cat gag aat gga gaa gaa gga tct ttg agt cta Ile Lys Val Phe Leu His Glu Asn Gly Glu Glu Gly Ser Leu Ser Leu 1480 1485 1490 1495	4636		
20	gca agt gtt att att tct gcc ctt tgt acc ctt aca agg cgt aac ctg Ala Ser Val Ile Ile Ser Ala Leu Cys Thr Leu Thr Arg Arg Asn Leu 1500 1505 1510	4684		
25	atg atg gaa ggt gca gcg tca agt gct gga gaa cag ctg gtt gat ctg Met Met Glu Gly Ala Ala Ser Ser Ala Gly Glu Gln Leu Val Asp Leu 1515 1520 1525	4732		
30	act tct cgg gat gga gcc tgg ttc ttg gag gaa ctc tgc agt atg agc Thr Ser Arg Asp Gly Ala Trp Phe Leu Glu Glu Leu Cys Ser Met Ser 1530 1535 1540	4780		
35	gga aac gtc acc tgc ttg gtt cag tta ctg aag cag tgc cac ctg gtg Gly Asn Val Thr Cys Leu Val Gln Leu Leu Lys Gln Cys His Leu Val 1545 1550 1555	4828		
40	cca cag gac tta gat atc ccg aac ccc atg gaa gcg tct gag aca gtt Pro Gln Asp Leu Asp Ile Pro Asn Pro Met Glu Ala Ser Glu Thr Val 1560 1565 1570 1575	4876		
45	cac tta gcc aat gga gtg tat acc tca ctt cag gaa ttg aat tgc aat His Leu Ala Asn Gly Val Tyr Thr Ser Leu Gln Glu Leu Asn Ser Asn 1580 1585 1590	4924		
50	ttc cgg caa atc ata ttt cca gaa gca ctt cga tgt tta atg aaa ggg Phe Arg Gln Ile Ile Phe Pro Glu Ala Leu Arg Cys Leu Met Lys Gly 1595 1600 1605	4972		
55	gaa tac acg tta gaa agt atg ctg cat gaa ctg gac ggt ctt att gag Glu Tyr Thr Leu Glu Ser Met Leu His Glu Leu Asp Gly Leu Ile Glu 1610 1615 1620	5020		
60	cag acc acc gat ggc gtt ccc ctg cag act cta gtg gaa tct ctt cag Gln Thr Thr Asp Gly Val Pro Leu Gln Thr Leu Val Glu Ser Leu Gln 1625 1630 1635	5068		

EP 1 231 264 A1

5	gcc tac tta aga aac gca gct atg gga ctg gaa gaa gaa aca cat gct Ala Tyr Leu Arg Asn Ala Ala Met Gly Leu Glu Glu Thr His Ala 1640 1645 1650 1655	5116
10	cat tac atc gat gtt gcc aga cta cta cat gct cag tac ggt gaa tta His Tyr Ile Asp Val Ala Arg Leu Leu His Ala Gln Tyr Gly Glu Leu 1660 1665 1670	5164
15	atc caa ccg aga aat ggt tca gtt gat gaa aca ccc aaa atg tca gct Ile Gln Pro Arg Asn Gly Ser Val Asp Glu Thr Pro Lys Met Ser Ala 1675 1680 1685	5212
20	ggc cag atg ctt ttg gta gca ttc gat ggc atg ttt gct caa gtt gaa Gly Gln Met Leu Leu Val Ala Phe Asp Gly Met Phe Ala Gln Val Glu 1690 1695 1700	5260
25	act gct ttc agc tta tta gtt gaa aag ttg aac aag atg gaa att ccc Thr Ala Phe Ser Leu Leu Val Glu Lys Leu Asn Lys Met Glu Ile Pro 1705 1710 1715	5308
30	ata gct tgg cga aag att gac atc ata agg gaa gcc agg agt act caa Ile Ala Trp Arg Lys Ile Asp Ile Ile Arg Glu Ala Arg Ser Thr Gln 1720 1725 1730 1735	5356
35	gtt aat ttt ttt gat gat gat aat cac cgg cag gtg cta gaa gag att Val Asn Phe Phe Asp Asp Asp Asn His Arg Gln Val Leu Glu Glu Ile 1740 1745 1750	5404
40	ttc ttt cta aaa aga cta cag act att aag gag ttc ttc agg ctc tgt Phe Phe Leu Lys Arg Leu Gln Thr Ile Lys Glu Phe Phe Arg Leu Cys 1755 1760 1765	5452
45	ggc acc ttt tct aaa aca ttg tca gga tca agt tca ctt gaa gat cag Gly Thr Phe Ser Lys Thr Leu Ser Gly Ser Ser Ser Leu Glu Asp Gln 1770 1775 1780	5500
50	aat act gtg aat ggg cct gta cag att gtc aat gtg aaa acc ctt ttt Asn Thr Val Asn Gly Pro Val Gln Ile Val Asn Val Lys Thr Leu Phe 1785 1790 1795	5548
55	aga aac tct tgt ttc agt gaa gac caa atg gcc aaa cct atc aag gca Arg Asn Ser Cys Phe Ser Glu Asp Gln Met Ala Lys Pro Ile Lys Ala 1800 1805 1810 1815	5596
	ttc aca gct gac ttt gtg agg cag ctc ttg ata ggg cta ccc aac caa Phe Thr Ala Asp Phe Val Arg Gln Leu Leu Ile Gly Leu Pro Asn Gln 1820 1825 1830	5644

EP 1 231 264 A1

	gcc ctc gga ctc aca ctg tgc agt ttt atc agt gct ctg ggt gta gac	5692
	Ala Leu Gly Leu Thr Leu Cys Ser Phe Ile Ser Ala Leu Gly Val Asp	
5	1835 1840 1845	
	atc att gct caa gta gag gca aag gac ttt ggt gcc gaa agc aaa gtt	5740
	Ile Ile Ala Gln Val Glu Ala Lys Asp Phe Gly Ala Glu Ser Lys Val	
	1850 1855 1860	
10	tct gtt gat gat ctc tgt aag aaa gcg gtg gaa cat aac atc cag ata	5788
	Ser Val Asp Asp Leu Cys Lys Lys Ala Val Glu His Asn Ile Gln Ile	
	1865 1870 1875	
15	ggg aag ttc tct cag ctg gtt atg aac agg gca act gtg tta gca agt	5836
	Gly Lys Phe Ser Gln Leu Val Met Asn Arg Ala Thr Val Leu Ala Ser	
	1880 1885 1890 1895	
20	tct tac gac act gcc tgg aag aag cat gac ttg gtg cga agg cta gaa	5884
	Ser Tyr Asp Thr Ala Trp Lys Lys His Asp Leu Val Arg Arg Leu Glu	
	1900 1905 1910	
25	acc agt att tct tct tgt aag aca agc ctg cag cgg gtt cag ctg cat	5932
	Thr Ser Ile Ser Ser Cys Lys Thr Ser Leu Gln Arg Val Gln Leu His	
	1915 1920 1925	
30	att gcc atg ttt cag tgg caa cat gaa gat cta ctt atc aat aga cca	5980
	Ile Ala Met Phe Gln Trp Gln His Glu Asp Leu Leu Ile Asn Arg Pro	
	1930 1935 1940	
35	caa gcc atg tca gtc aca cct ccc cca cgg tct gct atc cta acc agc	6028
	Gln Ala Met Ser Val Thr Pro Pro Pro Arg Ser Ala Ile Leu Thr Ser	
	1945 1950 1955	
40	atg aaa aag aag ctg cat acc ctg agc cag att gaa act tct att gcg	6076
	Met Lys Lys Lys Leu His Thr Leu Ser Gln Ile Glu Thr Ser Ile Ala	
	1960 1965 1970 1975	
45	aca gtt cag gag aag cta gct gca ctt gaa tca agt att gaa cag cga	6124
	Thr Val Gln Glu Lys Leu Ala Ala Leu Glu Ser Ser Ile Glu Gln Arg	
	1980 1985 1990	
50	ctc aag tgg gca ggt ggt gcc aac cct gca ttg gcc cct gta cta caa	6172
	Leu Lys Trp Ala Gly Gly Ala Asn Pro Ala Leu Ala Pro Val Leu Gln	
	1995 2000 2005	
55	gat ttt gaa gca acg ata gct gaa aga aga aat ctt gtc ctt aaa gag	6220
	Asp Phe Glu Ala Thr Ile Ala Glu Arg Arg Asn Leu Val Leu Lys Glu	
	2010 2015 2020	
	agc caa aga gca agt cag gtc aca ttt ctc tgc agc aat atc att cat	6268

EP 1 231 264 A1

	Ser Gln Arg Ala Ser Gln Val Thr Phe Leu Cys Ser Asn Ile Ile His	
	2025 2030 2035	
5	ttt gaa agt tta cga aca aga act gca gaa gcc tta aac ctg gat gcg Phe Glu Ser Leu Arg Thr Arg Thr Ala Glu Ala Leu Asn Leu Asp Ala	6316
	2040 2045 2050 2055	
10	gcg tta ttt gaa cta atc aag cga tgt cag cag atg tgt tcg ttt gca Ala Leu Phe Glu Leu Ile Lys Arg Cys Gln Gln Met Cys Ser Phe Ala	6364
	2060 2065 2070	
15	tca cag ttt aac agt tca gtg tct gag tta gag ctt cgt tta tta cag Ser Gln Phe Asn Ser Ser Val Ser Glu Leu Glu Leu Arg Leu Leu Gln	6412
	2075 2080 2085	
20	aga gtg gac act ggt ctt gaa cat cct att gcc agc tct gaa tgg ctt Arg Val Asp Thr Gly Leu Glu His Pro Ile Gly Ser Ser Glu Trp Leu	6460
	2090 2095 2100	
25	ttg tca gca cac aaa cag ttg acc cag gat atg tct act cag agg gca Leu Ser Ala His Lys Gln Leu Thr Gln Asp Met Ser Thr Gln Arg Ala	6508
	2105 2110 2115	
30	att cag aca gag aaa gag cag cag ata gaa acg gtc tgt gaa aca att Ile Gln Thr Glu Lys Glu Gln Gln Ile Glu Thr Val Cys Glu Thr Ile	6556
	2120 2125 2130 2135	
35	cag aat ctg gtt gat aat ata aag act gtg ctc act ggt cat aac cga Gln Asn Leu Val Asp Asn Ile Lys Thr Val Leu Thr Gly His Asn Arg	6604
	2140 2145 2150	
40	cag ctt gga gat gtc aaa cat ctc ttg aaa gct atg gct aag gat gaa Gln Leu Gly Asp Val Lys His Leu Leu Lys Ala Met Ala Lys Asp Glu	6652
	2155 2160 2165	
45	gaa gct gct ctg gca gat ggt gaa gat gtt ccc tat gag aac agt gtt Glu Ala Ala Leu Ala Asp Gly Glu Asp Val Pro Tyr Glu Asn Ser Val	6700
	2170 2175 2180	
50	agg cag ttt ttg ggt gaa tat aaa tca tgg caa gac aac att caa aca Arg Gln Phe Leu Gly Glu Tyr Lys Ser Trp Gln Asp Asn Ile Gln Thr	6748
	2185 2190 2195	
55	gtt cta ttt aca tta gtc cag gct atg ggt cag gtt cga agt caa gaa Val Leu Phe Thr Leu Val Gln Ala Met Gly Gln Val Arg Ser Gln Glu	6796
	2200 2205 2210 2215	
60	cac gtt gaa atg ctc cag gaa atc act ccc acc ttg aaa gaa ctg aaa His Val Glu Met Leu Gln Glu Ile Thr Pro Thr Leu Lys Glu Leu Lys	6844

EP 1 231 264 A1

	2220	2225	2230			
5	aca caa agt cag agt atc tat aat aat tta gtg agt ttt gca tca ccc Thr Gln Ser Gln Ser Ile Tyr Asn Asn Leu Val Ser Phe Ala Ser Pro	2235	2240	2245	6892	
10	tta gtc acc gat gca aca aat gaa tgt tcg agt cca acg tca tct gct Leu Val Thr Asp Ala Thr Asn Glu Cys Ser Ser Pro Thr Ser Ser Ala	2250	2255	2260	6940	
15	act tat cag cca tcc ttc gct gca gca gtc cgg agt aac act ggc cag Thr Tyr Gln Pro Ser Phe Ala Ala Ala Val Arg Ser Asn Thr Gly Gln	2265	2270	2275	6988	
20	aag act cag cct gat gtc atg tca cag aat gct aga aag ctg atc cag Lys Thr Gln Pro Asp Val Met Ser Gln Asn Ala Arg Lys Leu Ile Gln	2280	2285	2290	2295	7036
25	aaa aat ctt gct aca tca gct gat act cca cca agc acc gtt cca gga Lys Asn Leu Ala Thr Ser Ala Asp Thr Pro Pro Ser Thr Val Pro Gly	2300	2305	2310	7084	
30	act ggc aag agt gtt gct tgt agt cct aaa aag gca gtc aga gac cct Thr Gly Lys Ser Val Ala Cys Ser Pro Lys Lys Ala Val Arg Asp Pro	2315	2320	2325	7132	
35	aaa act ggg aaa gcg gtg caa gag aga aac tcc tat gca gtg agt gtg Lys Thr Gly Lys Ala Val Gln Glu Arg Asn Ser Tyr Ala Val Ser Val	2330	2335	2340	7180	
40	tgg aag aga gtg aaa gcc aag tta gag ggc cga gat gtt gat ccg aat Trp Lys Arg Val Lys Ala Lys Leu Glu Gly Arg Asp Val Asp Pro Asn	2345	2350	2355	7228	
45	agg agg atg tca gtt gct gaa cag gtt gac tat gtc att aag gaa gca Arg Arg Met Ser Val Ala Glu Gln Val Asp Tyr Val Ile Lys Glu Ala	2360	2365	2370	2375	7276
50	act aat cta gat aac ttg gct cag ctg tat gaa ggt tgg aca gcc tgg Thr Asn Leu Asp Asn Leu Ala Gln Leu Tyr Glu Gly Trp Thr Ala Trp	2380	2385	2390	7324	
55	gtg tgaatggcaa gacagtagat gagtctggtt aagcgaaggtc agacatccac Val				7377	
	cagaatcaac tcagcctcag gcacccaaag ccacaccaca gtcggtggtg atgcaactgg				7437	
	gggcttactc tgaggaaacc taggaaatct cggtgcacta ggaagtgaat cccgcaggac				7497	

EP 1 231 264 A1

agctgcactc agggatacgc ccaacaccat ggcctgcaac cccaggggtca aggggtgaagg 7557  
 aaagcaagct caccgcctga acacggagat tgtctttctg ccacagaaca gcagcagacg 7617  
 5 tgtcgggagg ttagctgcgg aaagaaatcg ggatgccgcg gagcacagag tgatttgaa 7677  
 ctccattcca cctgaccctg tgtgtacaat ccaggaaaaa aacaaacccc actcagaaac 7737  
 10 agagaaaact ggggtcgcga agaaatcaca gccaaaggaag atttgatgca ttcagattct 7797  
 cgtgtaacac ttgttgcttg gcaacagtac tggttgggtt gaccagtaag tagaaaaagg 7857  
 ctaaaggcta tgcgatatga atttcagaaa tggactgaaa atggagagct atgtaacaga 7917  
 15 tactactacag tagaagaact tacttctgaa atgaaggga aaaaaccacc ccacgttcc 7977  
 ctactcctcc ccaccactta cccgttcccc ctttacctaa tctagtagat tagccattct 8037  
 20 tcaaattcac ttttatttca gtccttatat ttcataact tccgtctcga tgctgttaac 8097  
 aacttctgat aacatggaaa attcaaggat tgtttaagg tctgatgatc acacacaaaa 8157  
 25 tgtaattccg gttatttaag tcatttctgt gattctatca tgtacagttt ccagaattgt 8217  
 cactgtgcat tcaaaagtaa tgaatctaac agacatttga ttaaatgtac actccctttt 8277  
 gcttatagtg tgcatttttt ttggaggcca ttcaaatttt ccctcttctg tgatagctgt 8337  
 30 agtttctttc atagaaagta gctaataccag tgtaatcttt taccttttta aaaaccaaga 8397  
 tagagtatct attagagttt tacattgttg atgatagatt aacaataaag tgatgttctg 8457  
 35 gtggaggtag actgaaattt ttttaattca tgtttttcat ttgatacttt taatttacac 8517  
 ttagtaaatt aaaagtgtt taatttactt ggcatttttag gacatgtaca tgaaacagtg 8577  
 aaaatgagat ccaccaacat cttttattaa gttcagttat tagtctgtga agtgctttac 8637  
 40 tttttgcaca attttaatag cttgctattc agtaatacat tatagtgaat tcatgatcaa 8697  
 ggtttcctta aatttagcat tgcatttcag tactgactgt gtaagctaaa ttgctgatcc 8757  
 45 aaaataaaaa cccagactag aataggggtc ttaaaatcaa gtatcaatac aaaatagaac 8817  
 acaattaaaa tcttaattgt tggctgggca cagtggctca cgcctgtaat cccagactt 8877  
 tgggaggccg aggcgggcg atcatgaggt taggagagcg agaccatcct ggctaacacg 8937  
 50 gtgaaacccc gtctttacta aaatacaaaa aaaattagcc ggggtgtggtg gcgggcgcct 8997

55

EP 1 231 264 A1

gtagtcccag ctactcggga ggctgaggca ggagaatggc gtgaacccag gaggcggagc 9057

ttgcagtgag ccgagattgt gccactgcac tccagcctgg gcaacagagc tagactctgt 9117

gtcaaaaata aatgactaga t 9138

<210> 4

<211> 2392

<212> PRT

<213> Homo sapiens

<400> 4

Met Lys Lys Leu Leu Pro Asn Met Leu Ser Pro Asp Pro Arg Glu Leu  
1 5 10 15

Gln Lys Ser Ile Glu Val Gln Leu Leu Arg Ser Ser Val Cys Leu Ala  
20 25 30

Thr Ala Leu Asn Pro Ile Glu Gln Asp Gln Lys Trp Gln Ser Ile Thr  
35 40 45

Glu Asn Val Val Lys Tyr Leu Lys Gln Thr Ser Arg Ile Ala Ile Gly  
50 55 60

Pro Leu Arg Leu Ser Thr Leu Thr Val Ser Gln Ser Leu Pro Val Leu  
65 70 75 80

Ser Thr Leu Gln Leu Tyr Cys Ser Ser Ala Leu Glu Asn Thr Val Ser  
85 90 95

Asn Arg Leu Ser Thr Glu Asp Cys Leu Ile Pro Leu Phe Ser Glu Ala  
100 105 110

Leu Arg Ser Cys Lys Gln His Asp Val Arg Pro Trp Met Gln Ala Leu  
115 120 125

Arg Tyr Thr Met Tyr Gln Asn Gln Leu Leu Glu Lys Ile Lys Glu Gln  
130 135 140

Thr Val Pro Ile Arg Ser His Leu Met Glu Leu Gly Leu Thr Ala Ala  
145 150 155 160

Lys Phe Ala Arg Lys Arg Gly Asn Val Ser Leu Ala Thr Arg Leu Leu  
165 170 175

Ala Gln Cys Ser Glu Val Gln Leu Gly Lys Thr Thr Thr Ala Gln Asp  
180 185 190

EP 1 231 264 A1

	Leu Val Gln His Phe Lys Lys Leu Ser Thr Gln Gly Gln Val Asp Glu	
	195	200 205
5	Lys Trp Gly Pro Glu Leu Asp Ile Glu Lys Thr Lys Leu Leu Tyr Thr	
	210	215 220
	Ala Gly Gln Ser Thr His Ala Met Glu Met Leu Ser Ser Cys Ala Ile	
10	225	230 235 240
	Ser Phe Cys Lys Ser Val Lys Ala Glu Tyr Ala Val Ala Lys Ser Ile	
	245	250 255
15	Leu Thr Leu Ala Lys Trp Ile Gln Ala Glu Trp Lys Glu Ile Ser Gly	
	260	265 270
	Gln Leu Lys Gln Val Tyr Arg Ala Gln His Gln Gln Asn Phe Thr Gly	
	275	280 285
20	Leu Ser Thr Leu Ser Lys Asn Ile Leu Thr Leu Ile Glu Leu Pro Ser	
	290	295 300
	Val Asn Thr Met Glu Glu Glu Tyr Pro Arg Ile Glu Ser Glu Ser Thr	
25	305	310 315 320
	Val His Ile Gly Val Gly Glu Pro Asp Phe Ile Leu Gly Gln Leu Tyr	
	325	330 335
30	His Leu Ser Ser Val Gln Ala Pro Glu Val Ala Lys Ser Trp Ala Ala	
	340	345 350
	Leu Ala Ser Trp Ala Tyr Arg Trp Gly Arg Lys Val Val Asp Asn Ala	
	355	360 365
35	Ser Gln Gly Glu Gly Val Arg Leu Leu Pro Arg Glu Lys Ser Glu Val	
	370	375 380
	Gln Asn Leu Leu Pro Asp Thr Ile Thr Glu Glu Glu Lys Glu Arg Ile	
40	385	390 395 400
	Tyr Gly Ile Leu Gly Gln Ala Val Cys Arg Pro Ala Gly Ile Gln Asp	
	405	410 415
45	Glu Asp Ile Thr Leu Gln Ile Thr Glu Ser Glu Asp Asn Glu Glu Asp	
	420	425 430
	Asp Met Val Asp Val Ile Trp Arg Gln Leu Ile Ser Ser Cys Pro Trp	
50	435	440 445

55



EP 1 231 264 A1

	Leu Ser Glu Leu Asp Glu Ser Ala Thr Glu Gly Val Ile Lys Val Trp	
	450	455 460
5	Arg Lys Val Val Asp Arg Ile Phe Ser Leu Tyr Lys Leu Ser Cys Ser	
	465	470 475 480
	Ala Tyr Phe Thr Phe Leu Lys Leu Asn Ala Gly Gln Ile Pro Leu Asp	
10		485 490 495
	Glu Asp Asp Pro Arg Leu His Leu Ser His Arg Val Glu Gln Ser Thr	
		500 505 510
15	Asp Asp Met Ile Val Met Ala Thr Leu Arg Leu Leu Arg Leu Leu Val	
	515	520 525
	Lys His Ala Gly Glu Leu Arg Gln Tyr Leu Glu His Gly Leu Glu Thr	
	530	535 540
20	Thr Pro Thr Ala Pro Trp Arg Gly Ile Ile Pro Gln Leu Phe Ser Arg	
	545	550 555 560
	Leu Asn His Pro Glu Val Tyr Val Arg Gln Ser Ile Cys Asn Leu Leu	
25		565 570 575
	Cys Arg Val Ala Gln Asp Ser Pro His Leu Ile Leu Tyr Pro Ala Ile	
		580 585 590
30	Val Gly Thr Ile Ser Leu Ser Ser Glu Ser Gln Ala Ser Gly Asn Lys	
	595	600 605
	Phe Ser Thr Ala Ile Pro Thr Leu Leu Gly Asn Ile Gln Gly Glu Glu	
	610	615 620
35	Leu Leu Val Ser Glu Cys Glu Gly Gly Ser Pro Pro Ala Ser Gln Asp	
	625	630 635 640
	Ser Asn Lys Asp Glu Pro Lys Ser Gly Leu Asn Glu Asp Gln Ala Met	
40		645 650 655
	Met Gln Asp Cys Tyr Ser Lys Ile Val Asp Lys Leu Ser Ser Ala Asn	
	660	665 670
45	Pro Thr Met Val Leu Gln Val Gln Met Leu Val Ala Glu Leu Arg Arg	
	675	680 685
	Val Thr Val Leu Trp Asp Glu Leu Trp Leu Gly Val Leu Leu Gln Gln	
	690	695 700
50	His Met Tyr Val Leu Arg Arg Ile Gln Gln Leu Glu Asp Glu Val Lys	
55		

EP 1 231 264 A1

	705		710		715		720
5	Arg Val Gln Asn Asn Thr Leu Arg Lys Glu Glu Lys Ile Ala Ile	725		730		735	
	Met Arg Glu Lys His Thr Ala Leu Met Lys Pro Ile Val Phe Ala Leu	740		745		750	
10	Glu His Val Arg Ser Ile Thr Ala Ala Pro Ala Glu Thr Pro His Glu	755		760		765	
	Lys Trp Phe Gln Asp Asn Tyr Gly Asp Ala Ile Glu Asn Ala Leu Glu	770		775		780	
15	Lys Leu Lys Thr Pro Leu Asn Pro Ala Lys Pro Gly Ser Ser Trp Ile	785		790		795	800
20	Pro Phe Lys Glu Ile Met Leu Asn Leu Gln Gln Arg Ala Gln Lys Arg	805		810		815	
	Ala Ser Tyr Ile Leu Arg Leu Glu Glu Ile Ser Pro Trp Leu Ala Ala	820		825		830	
25	Met Thr Asn Thr Glu Ile Ala Leu Pro Gly Glu Val Ser Ala Arg Asp	835		840		845	
	Thr Val Thr Ile His Ser Val Gly Gly Thr Ile Thr Ile Leu Pro Thr	850		855		860	
30	Lys Thr Lys Pro Lys Lys Leu Leu Phe Leu Gly Ser Asp Gly Lys Ser	865		870		875	880
	Tyr Pro Tyr Leu Phe Lys Gly Leu Glu Asp Leu His Leu Asp Glu Arg	885		890		895	
35	Ile Met Gln Phe Leu Ser Ile Val Asn Thr Met Phe Ala Thr Ile Asn	900		905		910	
40	Arg Gln Glu Thr Pro Arg Phe His Ala Arg His Tyr Ser Val Thr Pro	915		920		925	
	Leu Gly Thr Arg Ser Gly Leu Ile Gln Trp Val Asp Gly Ala Thr Pro	930		935		940	
45	Leu Phe Gly Leu Tyr Lys Arg Trp Gln Gln Arg Glu Ala Ala Leu Gln	945		950		955	960
	Ala Gln Lys Ala Gln Asp Ser Tyr Gln Thr Pro Gln Asn Pro Gly Ile	965		970		975	
50							
55							

EP 1 231 264 A1

Val Pro Arg Pro Ser Glu Leu Tyr Tyr Ser Lys Ile Gly Pro Ala Leu  
980 985 990

5 Lys Thr Val Gly Leu Ser Leu Asp Val Ser Arg Arg Asp Trp Pro Leu  
995 1000 1005

His Val Met Lys Ala Val Leu Glu Glu Leu Met Glu Ala Thr Pro Pro  
1010 1015 1020

10 Asn Leu Leu Ala Lys Glu Leu Trp Ser Ser Cys Thr Thr Pro Asp Glu  
025 1030 1035 1040

15 Trp Trp Arg Val Thr Gln Ser Tyr Ala Arg Ser Thr Ala Val Met Ser  
1045 1050 1055

Met Val Gly Tyr Ile Ile Gly Leu Gly Asp Arg His Leu Asp Asn Val  
1060 1065 1070

20 Leu Ile Asp Met Thr Thr Gly Glu Val Val His Ile Asp Tyr Asn Val  
1075 1080 1085

Cys Phe Glu Lys Gly Lys Ser Leu Arg Val Pro Glu Lys Val Pro Phe  
25 1090 1095 1100

Arg Met Thr Gln Asn Ile Glu Thr Ala Leu Gly Val Thr Gly Val Glu  
105 1110 1115 1120

30 Gly Val Phe Arg Leu Ser Cys Glu Gln Val Leu His Ile Met Arg Arg  
1125 1130 1135

Gly Arg Glu Thr Leu Leu Thr Leu Leu Glu Ala Phe Val Tyr Asp Pro  
1140 1145 1150

35 Leu Val Asp Trp Thr Ala Gly Gly Glu Ala Gly Phe Ala Gly Ala Val  
1155 1160 1165

Tyr Gly Gly Gly Gly Gln Gln Ala Glu Ser Lys Gln Ser Lys Arg Glu  
40 1170 1175 1180

Met Glu Arg Glu Ile Thr Arg Ser Leu Phe Ser Ser Arg Val Ala Glu  
185 1190 1195 1200

45 Ile Lys Val Asn Trp Phe Lys Asn Arg Asp Glu Met Leu Val Val Leu  
1205 1210 1215

Pro Lys Leu Asp Gly Ser Leu Asp Glu Tyr Leu Ser Leu Gln Glu Gln  
50 1220 1225 1230

Leu Thr Asp Val Glu Lys Leu Gln Gly Lys Leu Leu Glu Glu Ile Glu

55

EP 1 231 264 A1

	1235	1240	1245
5	Phe Leu Glu Gly Ala Glu Gly Val Asp His Pro Ser His Thr Leu Gln 1250 1255 1260		
	His Arg Tyr Ser Glu His Thr Gln Leu Gln Thr Gln Gln Arg Ala Val 265 1270 1275 1280		
10	Gln Glu Ala Ile Gln Val Lys Leu Asn Glu Phe Glu Gln Trp Ile Thr 1285 1290 1295		
15	His Tyr Gln Ala Ala Phe Asn Asn Leu Glu Ala Thr Gln Leu Ala Ser 1300 1305 1310		
	Leu Leu Gln Glu Ile Ser Thr Gln Met Asp Leu Gly Pro Pro Ser Tyr 1315 1320 1325		
20	Val Pro Ala Thr Ala Phe Leu Gln Asn Ala Gly Gln Ala His Leu Ile 1330 1335 1340		
	Ser Gln Cys Glu Gln Leu Glu Gly Glu Val Gly Ala Leu Leu Gln Gln 345 1350 1355 1360		
25	Arg Arg Ser Val Leu Arg Gly Cys Leu Glu Gln Leu His His Tyr Ala 1365 1370 1375		
30	Thr Val Ala Leu Gln Tyr Pro Lys Ala Ile Phe Gln Lys His Arg Ile 1380 1385 1390		
	Glu Gln Trp Lys Thr Trp Met Glu Glu Leu Ile Cys Asn Thr Thr Val 1395 1400 1405		
35	Glu Arg Cys Gln Glu Leu Tyr Arg Lys Tyr Glu Met Gln Tyr Ala Pro 1410 1415 1420		
40	Gln Pro Pro Pro Thr Val Cys Gln Phe Ile Thr Ala Thr Glu Met Thr 425 1430 1435 1440		
	Leu Gln Arg Tyr Ala Ala Asp Ile Asn Ser Arg Leu Ile Arg Gln Val 1445 1450 1455		
45	Glu Arg Leu Lys Gln Glu Ala Val Thr Val Pro Val Cys Glu Asp Gln 1460 1465 1470		
	Leu Lys Glu Ile Glu Arg Cys Ile Lys Val Phe Leu His Glu Asn Gly 1475 1480 1485		
50	Glu Glu Gly Ser Leu Ser Leu Ala Ser Val Ile Ile Ser Ala Leu Cys 1490 1495 1500		

55

EP 1 231 264 A1

	Thr	Leu	Thr	Arg	Arg	Asn	Leu	Met	Met	Glu	Gly	Ala	Ala	Ser	Ser	Ala	
	505					1510					1515					1520	
5	Gly	Glu	Gln	Leu	Val	Asp	Leu	Thr	Ser	Arg	Asp	Gly	Ala	Trp	Phe	Leu	
					1525					1530					1535		
	Glu	Glu	Leu	Cys	Ser	Met	Ser	Gly	Asn	Val	Thr	Cys	Leu	Val	Gln	Leu	
10				1540					1545					1550			
	Leu	Lys	Gln	Cys	His	Leu	Val	Pro	Gln	Asp	Leu	Asp	Ile	Pro	Asn	Pro	
		1555						1560					1565				
15	Met	Glu	Ala	Ser	Glu	Thr	Val	His	Leu	Ala	Asn	Gly	Val	Tyr	Thr	Ser	
	1570						1575					1580					
	Leu	Gln	Glu	Leu	Asn	Ser	Asn	Phe	Arg	Gln	Ile	Ile	Phe	Pro	Glu	Ala	
20	585				1590					1595				1600			
	Leu	Arg	Cys	Leu	Met	Lys	Gly	Glu	Tyr	Thr	Leu	Glu	Ser	Met	Leu	His	
				1605					1610					1615			
25	Glu	Leu	Asp	Gly	Leu	Ile	Glu	Gln	Thr	Thr	Asp	Gly	Val	Pro	Leu	Gln	
		1620						1625					1630				
	Thr	Leu	Val	Glu	Ser	Leu	Gln	Ala	Tyr	Leu	Arg	Asn	Ala	Ala	Met	Gly	
		1635						1640					1645				
30	Leu	Glu	Glu	Glu	Thr	His	Ala	His	Tyr	Ile	Asp	Val	Ala	Arg	Leu	Leu	
	1650					1655					1660						
	His	Ala	Gln	Tyr	Gly	Glu	Leu	Ile	Gln	Pro	Arg	Asn	Gly	Ser	Val	Asp	
35	665				1670					1675				1680			
	Glu	Thr	Pro	Lys	Met	Ser	Ala	Gly	Gln	Met	Leu	Leu	Val	Ala	Phe	Asp	
				1685					1690					1695			
40	Gly	Met	Phe	Ala	Gln	Val	Glu	Thr	Ala	Phe	Ser	Leu	Leu	Val	Glu	Lys	
		1700						1705					1710				
	Leu	Asn	Lys	Met	Glu	Ile	Pro	Ile	Ala	Trp	Arg	Lys	Ile	Asp	Ile	Ile	
		1715						1720					1725				
45	Arg	Glu	Ala	Arg	Ser	Thr	Gln	Val	Asn	Phe	Phe	Asp	Asp	Asp	Asn	His	
	1730					1735						1740					
	Arg	Gln	Val	Leu	Glu	Glu	Ile	Phe	Phe	Leu	Lys	Arg	Leu	Gln	Thr	Ile	
50	745				1750					1755				1760			

EP 1 231 264 A1

Lys Glu Phe Phe Arg Leu Cys Gly Thr Phe Ser Lys Thr Leu Ser Gly  
 1765 1770 1775  
 5 Ser Ser Ser Leu Glu Asp Gln Asn Thr Val Asn Gly Pro Val Gln Ile  
 1780 1785 1790  
 Val Asn Val Lys Thr Leu Phe Arg Asn Ser Cys Phe Ser Glu Asp Gln  
 1795 1800 1805  
 10 Met Ala Lys Pro Ile Lys Ala Phe Thr Ala Asp Phe Val Arg Gln Leu  
 1810 1815 1820  
 Leu Ile Gly Leu Pro Asn Gln Ala Leu Gly Leu Thr Leu Cys Ser Phe  
 825 1830 1835 1840  
 Ile Ser Ala Leu Gly Val Asp Ile Ile Ala Gln Val Glu Ala Lys Asp  
 1845 1850 1855  
 20 Phe Gly Ala Glu Ser Lys Val Ser Val Asp Asp Leu Cys Lys Lys Ala  
 1860 1865 1870  
 Val Glu His Asn Ile Gln Ile Gly Lys Phe Ser Gln Leu Val Met Asn  
 1875 1880 1885  
 25 Arg Ala Thr Val Leu Ala Ser Ser Tyr Asp Thr Ala Trp Lys Lys His  
 1890 1895 1900  
 Asp Leu Val Arg Arg Leu Glu Thr Ser Ile Ser Ser Cys Lys Thr Ser  
 905 1910 1915 1920  
 Leu Gln Arg Val Gln Leu His Ile Ala Met Phe Gln Trp Gln His Glu  
 1925 1930 1935  
 35 Asp Leu Leu Ile Asn Arg Pro Gln Ala Met Ser Val Thr Pro Pro Pro  
 1940 1945 1950  
 Arg Ser Ala Ile Leu Thr Ser Met Lys Lys Lys Leu His Thr Leu Ser  
 1955 1960 1965  
 40 Gln Ile Glu Thr Ser Ile Ala Thr Val Gln Glu Lys Leu Ala Ala Leu  
 1970 1975 1980  
 Glu Ser Ser Ile Glu Gln Arg Leu Lys Trp Ala Gly Gly Ala Asn Pro  
 985 1990 1995 2000  
 Ala Leu Ala Pro Val Leu Gln Asp Phe Glu Ala Thr Ile Ala Glu Arg  
 2005 2010 2015  
 50 Arg Asn Leu Val Leu Lys Glu Ser Gln Arg Ala Ser Gln Val Thr Phe

55

EP 1 231 264 A1

	2020	2025	2030
5	Leu Cys Ser Asn Ile Ile His 2035	Phe Glu Ser Leu Arg Thr Arg Thr Ala 2040	
	Glu Ala Leu Asn Leu Asp Ala Ala Leu Phe Glu Leu Ile Lys Arg Cys 2050		2060
10	Gln Gln Met Cys Ser Phe Ala Ser Gln Phe Asn Ser Ser Val Ser Glu 065	2070	2075 2080
	Leu Glu Leu Arg Leu Leu Gln Arg Val Asp Thr Gly Leu Glu His Pro 2085	2090	2095
15	Ile Gly Ser Ser Glu Trp Leu Leu Ser Ala His Lys Gln Leu Thr Gln 2100	2105	2110
20	Asp Met Ser Thr Gln Arg Ala Ile Gln Thr Glu Lys Glu Gln Gln Ile 2115	2120	2125
	Glu Thr Val Cys Glu Thr Ile Gln Asn Leu Val Asp Asn Ile Lys Thr 2130	2135	2140
25	Val Leu Thr Gly His Asn Arg Gln Leu Gly Asp Val Lys His Leu Leu 145	2150	2155 2160
	Lys Ala Met Ala Lys Asp Glu Glu Ala Ala Leu Ala Asp Gly Glu Asp 2165	2170	2175
30	Val Pro Tyr Glu Asn Ser Val Arg Gln Phe Leu Gly Glu Tyr Lys Ser 2180	2185	2190
35	Trp Gln Asp Asn Ile Gln Thr Val Leu Phe Thr Leu Val Gln Ala Met 2195	2200	2205
	Gly Gln Val Arg Ser Gln Glu His Val Glu Met Leu Gln Glu Ile Thr 2210	2215	2220
40	Pro Thr Leu Lys Glu Leu Lys Thr Gln Ser Gln Ser Ile Tyr Asn Asn 225	2230	2235 2240
	Leu Val Ser Phe Ala Ser Pro Leu Val Thr Asp Ala Thr Asn Glu Cys 2245	2250	2255
45	Ser Ser Pro Thr Ser Ser Ala Thr Tyr Gln Pro Ser Phe Ala Ala Ala 2260	2265	2270
50	Val Arg Ser Asn Thr Gly Gln Lys Thr Gln Pro Asp Val Met Ser Gln 2275	2280	2285

55

EP 1 231 264 A1

Asn Ala Arg Lys Leu Ile Gln Lys Asn Leu Ala Thr Ser Ala Asp Thr  
2290 2295 2300

5 Pro Pro Ser Thr Val Pro Gly Thr Gly Lys Ser Val Ala Cys Ser Pro  
305 2310 2315 2320

Lys Lys Ala Val Arg Asp Pro Lys Thr Gly Lys Ala Val Gln Glu Arg  
10 2325 2330 2335

Asn Ser Tyr Ala Val Ser Val Trp Lys Arg Val Lys Ala Lys Leu Glu  
2340 2345 2350

15 Gly Arg Asp Val Asp Pro Asn Arg Arg Met Ser Val Ala Glu Gln Val  
2355 2360 2365

Asp Tyr Val Ile Lys Glu Ala Thr Asn Leu Asp Asn Leu Ala Gln Leu  
2370 2375 2380

20 Tyr Glu Gly Trp Thr Ala Trp Val  
385 2390

25 <210> 5  
<211> 2392  
<212> PRT  
<213> Artificial Sequence

30 <220>  
<223> Description of Artificial Sequence: kinase  
inactive BLIP protein

35 <400> 5  
Met Lys Lys Leu Leu Pro Asn Met Leu Ser Pro Asp Pro Arg Glu Leu  
1 5 10 15

Gln Lys Ser Ile Glu Val Gln Leu Leu Arg Ser Ser Val Cys Leu Ala  
20 25 30

40 Thr Ala Leu Asn Pro Ile Glu Gln Asp Gln Lys Trp Gln Ser Ile Thr  
35 40 45

45 Glu Asn Val Val Lys Tyr Leu Lys Gln Thr Ser Arg Ile Ala Ile Gly  
50 55 60

Pro Leu Arg Leu Ser Thr Leu Thr Val Ser Gln Ser Leu Pro Val Leu  
65 70 75 80

50 Ser Thr Leu Gln Leu Tyr Cys Ser Ser Ala Leu Glu Asn Thr Val Ser

55



EP 1 231 264 A1

	85	90	95
5	Asn Arg Leu Ser Thr Glu Asp Cys Leu Ile Pro Leu Phe Ser Glu Ala 100 105 110		
	Leu Arg Ser Cys Lys Gln His Asp Val Arg Pro Trp Met Gln Ala Leu 115 120 125		
10	Arg Tyr Thr Met Tyr Gln Asn Gln Leu Leu Glu Lys Ile Lys Glu Gln 130 135 140		
	Thr Val Pro Ile Arg Ser His Leu Met Glu Leu Gly Leu Thr Ala Ala 145 150 155 160		
15	Lys Phe Ala Arg Lys Arg Gly Asn Val Ser Leu Ala Thr Arg Leu Leu 165 170 175		
	Ala Gln Cys Ser Glu Val Gln Leu Gly Lys Thr Thr Thr Ala Gln Asp 180 185 190		
20	Leu Val Gln His Phe Lys Lys Leu Ser Thr Gln Gly Gln Val Asp Glu 195 200 205		
25	Lys Trp Gly Pro Glu Leu Asp Ile Glu Lys Thr Lys Leu Leu Tyr Thr 210 215 220		
	Ala Gly Gln Ser Thr His Ala Met Glu Met Leu Ser Ser Cys Ala Ile 225 230 235 240		
30	Ser Phe Cys Lys Ser Val Lys Ala Glu Tyr Ala Val Ala Lys Ser Ile 245 250 255		
	Leu Thr Leu Ala Lys Trp Ile Gln Ala Glu Trp Lys Glu Ile Ser Gly 260 265 270		
35	Gln Leu Lys Gln Val Tyr Arg Ala Gln His Gln Gln Asn Phe Thr Gly 275 280 285		
40	Leu Ser Thr Leu Ser Lys Asn Ile Leu Thr Leu Ile Glu Leu Pro Ser 290 295 300		
	Val Asn Thr Met Glu Glu Glu Tyr Pro Arg Ile Glu Ser Glu Ser Thr 305 310 315 320		
45	Val His Ile Gly Val Gly Glu Pro Asp Phe Ile Leu Gly Gln Leu Tyr 325 330 335		
50	His Leu Ser Ser Val Gln Ala Pro Glu Val Ala Lys Ser Trp Ala Ala 340 345 350		

55

EP 1 231 264 A1

	Leu	Ala	Ser	Trp	Ala	Tyr	Arg	Trp	Gly	Arg	Lys	Val	Val	Asp	Asn	Ala	
			355					360						365			
5	Ser	Gln	Gly	Glu	Gly	Val	Arg	Leu	Leu	Pro	Arg	Glu	Lys	Ser	Glu	Val	
			370				375					380					
	Gln	Asn	Leu	Leu	Pro	Asp	Thr	Ile	Thr	Glu	Glu	Glu	Lys	Glu	Arg	Ile	
10			385			390					395					400	
	Tyr	Gly	Ile	Leu	Gly	Gln	Ala	Val	Cys	Arg	Pro	Ala	Gly	Ile	Gln	Asp	
				405						410					415		
	Glu	Asp	Ile	Thr	Leu	Gln	Ile	Thr	Glu	Ser	Glu	Asp	Asn	Glu	Glu	Asp	
15				420					425					430			
	Asp	Met	Val	Asp	Val	Ile	Trp	Arg	Gln	Leu	Ile	Ser	Ser	Cys	Pro	Trp	
			435					440					445				
20	Leu	Ser	Glu	Leu	Asp	Glu	Ser	Ala	Thr	Glu	Gly	Val	Ile	Lys	Val	Trp	
			450			455						460					
	Arg	Lys	Val	Val	Asp	Arg	Ile	Phe	Ser	Leu	Tyr	Lys	Leu	Ser	Cys	Ser	
25			465			470					475					480	
	Ala	Tyr	Phe	Thr	Phe	Leu	Lys	Leu	Asn	Ala	Gly	Gln	Ile	Pro	Leu	Asp	
				485						490					495		
30	Glu	Asp	Asp	Pro	Arg	Leu	His	Leu	Ser	His	Arg	Val	Glu	Gln	Ser	Thr	
				500					505					510			
	Asp	Asp	Met	Ile	Val	Met	Ala	Thr	Leu	Arg	Leu	Leu	Arg	Leu	Leu	Val	
			515				520						525				
35	Lys	His	Ala	Gly	Glu	Leu	Arg	Gln	Tyr	Leu	Glu	His	Gly	Leu	Glu	Thr	
			530				535					540					
	Thr	Pro	Thr	Ala	Pro	Trp	Arg	Gly	Ile	Ile	Pro	Gln	Leu	Phe	Ser	Arg	
40				545			550				555					560	
	Leu	Asn	His	Pro	Glu	Val	Tyr	Val	Arg	Gln	Ser	Ile	Cys	Asn	Leu	Leu	
				565					570					575			
45	Cys	Arg	Val	Ala	Gln	Asp	Ser	Pro	His	Leu	Ile	Leu	Tyr	Pro	Ala	Ile	
				580					585					590			
	Val	Gly	Thr	Ile	Ser	Leu	Ser	Ser	Glu	Ser	Gln	Ala	Ser	Gly	Asn	Lys	
			595				600					605					
50	Phe	Ser	Thr	Ala	Ile	Pro	Thr	Leu	Leu	Gly	Asn	Ile	Gln	Gly	Glu	Glu	
55																	

EP 1 231 264 A1

	610	615	620
5	Leu Leu Val Ser Glu Cys Glu Gly Gly Ser Pro Pro Ala Ser Gln Asp 625 630 635 640		
	Ser Asn Lys Asp Glu Pro Lys Ser Gly Leu Asn Glu Asp Gln Ala Met 645 650 655		
10	Met Gln Asp Cys Tyr Ser Lys Ile Val Asp Lys Leu Ser Ser Ala Asn 660 665 670		
	Pro Thr Met Val Leu Gln Val Gln Met Leu Val Ala Glu Leu Arg Arg 675 680 685		
15	Val Thr Val Leu Trp Asp Glu Leu Trp Leu Gly Val Leu Leu Gln Gln 690 695 700		
	His Met Tyr Val Leu Arg Arg Ile Gln Gln Leu Glu Asp Glu Val Lys 705 710 715 720		
20	Arg Val Gln Asn Asn Asn Thr Leu Arg Lys Glu Glu Lys Ile Ala Ile 725 730 735		
	Met Arg Glu Lys His Thr Ala Leu Met Lys Pro Ile Val Phe Ala Leu 740 745 750		
25	Glu His Val Arg Ser Ile Thr Ala Ala Pro Ala Glu Thr Pro His Glu 755 760 765		
30	Lys Trp Phe Gln Asp Asn Tyr Gly Asp Ala Ile Glu Asn Ala Leu Glu 770 775 780		
	Lys Leu Lys Thr Pro Leu Asn Pro Ala Lys Pro Gly Ser Ser Trp Ile 785 790 795 800		
35	Pro Phe Lys Glu Ile Met Leu Asn Leu Gln Gln Arg Ala Gln Lys Arg 805 810 815		
40	Ala Ser Tyr Ile Leu Arg Leu Glu Glu Ile Ser Pro Trp Leu Ala Ala 820 825 830		
	Met Thr Asn Thr Glu Ile Ala Leu Pro Gly Glu Val Ser Ala Arg Asp 835 840 845		
45	Thr Val Thr Ile His Ser Val Gly Gly Thr Ile Thr Ile Leu Pro Thr 850 855 860		
	Lys Thr Lys Pro Lys Lys Leu Leu Phe Leu Gly Ser Asp Gly Lys Ser 865 870 875 880		
50			
55			

EP 1 231 264 A1

	Tyr	Pro	Tyr	Leu	Phe	Lys	Gly	Leu	Glu	Asp	Leu	His	Leu	Asp	Glu	Arg	
					885					890					895		
5	Ile	Met	Gln	Phe	Leu	Ser	Ile	Val	Asn	Thr	Met	Phe	Ala	Thr	Ile	Asn	
					900					905					910		
	Arg	Gln	Glu	Thr	Pro	Arg	Phe	His	Ala	Arg	His	Tyr	Ser	Val	Thr	Pro	
10					915				920					925			
	Leu	Gly	Thr	Arg	Ser	Gly	Leu	Ile	Gln	Trp	Val	Asp	Gly	Ala	Thr	Pro	
					930				935					940			
	Leu	Phe	Gly	Leu	Tyr	Lys	Arg	Trp	Gln	Gln	Arg	Glu	Ala	Ala	Leu	Gln	
15					945						955					960	
	Ala	Gln	Lys	Ala	Gln	Asp	Ser	Tyr	Gln	Thr	Pro	Gln	Asn	Pro	Gly	Ile	
					965					970						975	
20	Val	Pro	Arg	Pro	Ser	Glu	Leu	Tyr	Tyr	Ser	Lys	Ile	Gly	Pro	Ala	Leu	
					980					985					990		
	Lys	Thr	Val	Gly	Leu	Ser	Leu	Asp	Val	Ser	Arg	Arg	Asp	Trp	Pro	Leu	
25					995				1000					1005			
	His	Val	Met	Lys	Ala	Val	Leu	Glu	Glu	Leu	Met	Glu	Ala	Thr	Pro	Pro	
					1010					1015				1020			
	Asn	Leu	Leu	Ala	Lys	Glu	Leu	Trp	Ser	Ser	Cys	Thr	Thr	Pro	Asp	Glu	
30					1025						1035					1040	
	Trp	Trp	Arg	Val	Thr	Gln	Ser	Tyr	Ala	Arg	Ser	Thr	Ala	Val	Met	Ser	
35					1045						1050					1055	
	Met	Val	Gly	Tyr	Ile	Ile	Gly	Leu	Gly	Ala	Arg	His	Leu	Asp	Lys	Val	
					1060					1065					1070		
	Leu	Ile	Asp	Met	Thr	Thr	Gly	Glu	Val	Val	His	Ile	Asp	Tyr	Asn	Val	
40					1075					1080				1085			
	Cys	Phe	Glu	Lys	Gly	Lys	Ser	Leu	Arg	Val	Pro	Glu	Lys	Val	Pro	Phe	
					1090					1095				1100			
	Arg	Met	Thr	Gln	Asn	Ile	Glu	Thr	Ala	Leu	Gly	Val	Thr	Gly	Val	Glu	
45					1105					1110				1115		1120	
	Gly	Val	Phe	Arg	Leu	Ser	Cys	Glu	Gln	Val	Leu	His	Ile	Met	Arg	Arg	
50					1125					1130					1135		

55

EP 1 231 264 A1

Gly Arg Glu Thr Leu Leu Thr Leu Leu Glu Ala Phe Val Tyr Asp Pro  
 1140 1145 1150  
 5 Leu Val Asp Trp Thr Ala Gly Gly Glu Ala Gly Phe Ala Gly Ala Val  
 1155 1160 1165  
 Tyr Gly Gly Gly Gly Gln Gln Ala Glu Ser Lys Gln Ser Lys Arg Glu  
 1170 1175 1180  
 10 Met Glu Arg Glu Ile Thr Arg Ser Leu Phe Ser Ser Arg Val Ala Glu  
 1185 1190 1195 1200  
 Ile Lys Val Asn Trp Phe Lys Asn Arg Asp Glu Met Leu Val Val Leu  
 1205 1210 1215  
 Pro Lys Leu Asp Gly Ser Leu Asp Glu Tyr Leu Ser Leu Gln Glu Gln  
 1220 1225 1230  
 20 Leu Thr Asp Val Glu Lys Leu Gln Gly Lys Leu Leu Glu Glu Ile Glu  
 1235 1240 1245  
 Phe Leu Glu Gly Ala Glu Gly Val Asp His Pro Ser His Thr Leu Gln  
 1250 1255 1260  
 25 His Arg Tyr Ser Glu His Thr Gln Leu Gln Thr Gln Gln Arg Ala Val  
 1265 1270 1275 1280  
 Gln Glu Ala Ile Gln Val Lys Leu Asn Glu Phe Glu Gln Trp Ile Thr  
 1285 1290 1295  
 His Tyr Gln Ala Ala Phe Asn Asn Leu Glu Ala Thr Gln Leu Ala Ser  
 1300 1305 1310  
 35 Leu Leu Gln Glu Ile Ser Thr Gln Met Asp Leu Gly Pro Pro Ser Tyr  
 1315 1320 1325  
 Val Pro Ala Thr Ala Phe Leu Gln Asn Ala Gly Gln Ala His Leu Ile  
 1330 1335 1340  
 40 Ser Gln Cys Glu Gln Leu Glu Gly Glu Val Gly Ala Leu Leu Gln Gln  
 1345 1350 1355 1360  
 Arg Arg Ser Val Leu Arg Gly Cys Leu Glu Gln Leu His His Tyr Ala  
 1365 1370 1375  
 45 Thr Val Ala Leu Gln Tyr Pro Lys Ala Ile Phe Gln Lys His Arg Ile  
 1380 1385 1390  
 50 Glu Gln Trp Lys Thr Trp Met Glu Glu Leu Ile Cys Asn Thr Thr Val

55

EP 1 231 264 A1

	1395	1400	1405
5	Glu Arg Cys Gln Glu Leu Tyr Arg Lys Tyr Glu Met Gln Tyr Ala Pro 1410 1415 1420		
	Gln Pro Pro Pro Thr Val Cys Gln Phe Ile Thr Ala Thr Glu Met Thr 1425 1430 1435 1440		
10	Leu Gln Arg Tyr Ala Ala Asp Ile Asn Ser Arg Leu Ile Arg Gln Val 1445 1450 1455		
	Glu Arg Leu Lys Gln Glu Ala Val Thr Val Pro Val Cys Glu Asp Gln 1460 1465 1470		
15	Leu Lys Glu Ile Glu Arg Cys Ile Lys Val Phe Leu His Glu Asn Gly 1475 1480 1485		
	Glu Glu Gly Ser Leu Ser Leu Ala Ser Val Ile Ile Ser Ala Leu Cys 1490 1495 1500		
20	Thr Leu Thr Arg Arg Asn Leu Met Met Glu Gly Ala Ala Ser Ser Ala 1505 1510 1515 1520		
	Gly Glu Gln Leu Val Asp Leu Thr Ser Arg Asp Gly Ala Trp Phe Leu 1525 1530 1535		
	Glu Glu Leu Cys Ser Met Ser Gly Asn Val Thr Cys Leu Val Gln Leu 1540 1545 1550		
30	Leu Lys Gln Cys His Leu Val Pro Gln Asp Leu Asp Ile Pro Asn Pro 1555 1560 1565		
	Met Glu Ala Ser Glu Thr Val His Leu Ala Asn Gly Val Tyr Thr Ser 1570 1575 1580		
	Leu Gln Glu Leu Asn Ser Asn Phe Arg Gln Ile Ile Phe Pro Glu Ala 1585 1590 1595 1600		
40	Leu Arg Cys Leu Met Lys Gly Glu Tyr Thr Leu Glu Ser Met Leu His 1605 1610 1615		
	Glu Leu Asp Gly Leu Ile Glu Gln Thr Thr Asp Gly Val Pro Leu Gln 1620 1625 1630		
45	Thr Leu Val Glu Ser Leu Gln Ala Tyr Leu Arg Asn Ala Ala Met Gly 1635 1640 1645		
	Leu Glu Glu Glu Thr His Ala His Tyr Ile Asp Val Ala Arg Leu Leu 1650 1655 1660		
50			
55			

EP 1 231 264 A1

	His	Ala	Gln	Tyr	Gly	Glu	Leu	Ile	Gln	Pro	Arg	Asn	Gly	Ser	Val	Asp
	1665					1670					1675					1680
5	Glu	Thr	Pro	Lys	Met	Ser	Ala	Gly	Gln	Met	Leu	Leu	Val	Ala	Phe	Asp
					1685					1690						1695
	Gly	Met	Phe	Ala	Gln	Val	Glu	Thr	Ala	Phe	Ser	Leu	Leu	Val	Glu	Lys
10				1700					1705						1710	
	Leu	Asn	Lys	Met	Glu	Ile	Pro	Ile	Ala	Trp	Arg	Lys	Ile	Asp	Ile	Ile
		1715						1720					1725			
15	Arg	Glu	Ala	Arg	Ser	Thr	Gln	Val	Asn	Phe	Phe	Asp	Asp	Asp	Asn	His
		1730					1735						1740			
	Arg	Gln	Val	Leu	Glu	Glu	Ile	Phe	Phe	Leu	Lys	Arg	Leu	Gln	Thr	Ile
20		1745				1750					1755					1760
	Lys	Glu	Phe	Phe	Arg	Leu	Cys	Gly	Thr	Phe	Ser	Lys	Thr	Leu	Ser	Gly
				1765					1770						1775	
25	Ser	Ser	Ser	Leu	Glu	Asp	Gln	Asn	Thr	Val	Asn	Gly	Pro	Val	Gln	Ile
				1780					1785						1790	
	Val	Asn	Val	Lys	Thr	Leu	Phe	Arg	Asn	Ser	Cys	Phe	Ser	Glu	Asp	Gln
		1795						1800						1805		
30	Met	Ala	Lys	Pro	Ile	Lys	Ala	Phe	Thr	Ala	Asp	Phe	Val	Arg	Gln	Leu
		1810					1815						1820			
	Leu	Ile	Gly	Leu	Pro	Asn	Gln	Ala	Leu	Gly	Leu	Thr	Leu	Cys	Ser	Phe
35		1825				1830					1835					1840
	Ile	Ser	Ala	Leu	Gly	Val	Asp	Ile	Ile	Ala	Gln	Val	Glu	Ala	Lys	Asp
				1845					1850						1855	
40	Phe	Gly	Ala	Glu	Ser	Lys	Val	Ser	Val	Asp	Asp	Leu	Cys	Lys	Lys	Ala
			1860						1865						1870	
	Val	Glu	His	Asn	Ile	Gln	Ile	Gly	Lys	Phe	Ser	Gln	Leu	Val	Met	Asn
		1875					1880						1885			
45	Arg	Ala	Thr	Val	Leu	Ala	Ser	Ser	Tyr	Asp	Thr	Ala	Trp	Lys	Lys	His
		1890					1895					1900				
50	Asp	Leu	Val	Arg	Arg	Leu	Glu	Thr	Ser	Ile	Ser	Ser	Cys	Lys	Thr	Ser
	1905					1910					1915					1920

EP 1 231 264 A1

5 Leu Gln Arg Val Gln Leu His Ile Ala Met Phe Gln Trp Gln His Glu  
 1925 1930 1935  
 Asp Leu Leu Ile Asn Arg Pro Gln Ala Met Ser Val Thr Pro Pro Pro  
 1940 1945 1950  
 10 Arg Ser Ala Ile Leu Thr Ser Met Lys Lys Lys Leu His Thr Leu Ser  
 1955 1960 1965  
 Gln Ile Glu Thr Ser Ile Ala Thr Val Gln Glu Lys Leu Ala Ala Leu  
 1970 1975 1980  
 15 Glu Ser Ser Ile Glu Gln Arg Leu Lys Trp Ala Gly Gly Ala Asn Pro  
 1985 1990 1995 2000  
 Ala Leu Ala Pro Val Leu Gln Asp Phe Glu Ala Thr Ile Ala Glu Arg  
 2005 2010 2015  
 20 Arg Asn Leu Val Leu Lys Glu Ser Gln Arg Ala Ser Gln Val Thr Phe  
 2020 2025 2030  
 Leu Cys Ser Asn Ile Ile His Phe Glu Ser Leu Arg Thr Arg Thr Ala  
 2035 2040 2045  
 25 Glu Ala Leu Asn Leu Asp Ala Ala Leu Phe Glu Leu Ile Lys Arg Cys  
 2050 2055 2060  
 Gln Gln Met Cys Ser Phe Ala Ser Gln Phe Asn Ser Ser Val Ser Glu  
 2065 2070 2075 2080  
 30 Leu Glu Leu Arg Leu Leu Gln Arg Val Asp Thr Gly Leu Glu His Pro  
 2085 2090 2095  
 35 Ile Gly Ser Ser Glu Trp Leu Leu Ser Ala His Lys Gln Leu Thr Gln  
 2100 2105 2110  
 Asp Met Ser Thr Gln Arg Ala Ile Gln Thr Glu Lys Glu Gln Gln Ile  
 2115 2120 2125  
 40 Glu Thr Val Cys Glu Thr Ile Gln Asn Leu Val Asp Asn Ile Lys Thr  
 2130 2135 2140  
 45 Val Leu Thr Gly His Asn Arg Gln Leu Gly Asp Val Lys His Leu Leu  
 2145 2150 2155 2160  
 Lys Ala Met Ala Lys Asp Glu Glu Ala Ala Leu Ala Asp Gly Glu Asp  
 2165 2170 2175  
 50 Val Pro Tyr Glu Asn Ser Val Arg Gln Phe Leu Gly Glu Tyr Lys Ser  
 2180 2185 2190

55



# EP 1 231 264 A1

Trp Gln Asp Asn Ile Gln Thr Val Leu Phe Thr Leu Val Gln Ala Met  
 2195 2200 2205  
 5 Gly Gln Val Arg Ser Gln Glu His Val Glu Met Leu Gln Glu Ile Thr  
 2210 2215 2220  
 Pro Thr Leu Lys Glu Leu Lys Thr Gln Ser Gln Ser Ile Tyr Asn Asn  
 2225 2230 2235 2240  
 10 Leu Val Ser Phe Ala Ser Pro Leu Val Thr Asp Ala Thr Asn Glu Cys  
 2245 2250 2255  
 Ser Ser Pro Thr Ser Ser Ala Thr Tyr Gln Pro Ser Phe Ala Ala Ala  
 2260 2265 2270  
 15 Val Arg Ser Asn Thr Gly Gln Lys Thr Gln Pro Asp Val Met Ser Gln  
 2275 2280 2285  
 20 Asn Ala Arg Lys Leu Ile Gln Lys Asn Leu Ala Thr Ser Ala Asp Thr  
 2290 2295 2300  
 Pro Pro Ser Thr Val Pro Gly Thr Gly Lys Ser Val Ala Cys Ser Pro  
 2305 2310 2315 2320  
 25 Lys Lys Ala Val Arg Asp Pro Lys Thr Gly Lys Ala Val Gln Glu Arg  
 2325 2330 2335  
 Asn Ser Tyr Ala Val Ser Val Trp Lys Arg Val Lys Ala Lys Leu Glu  
 2340 2345 2350  
 30 Gly Arg Asp Val Asp Pro Asn Arg Arg Met Ser Val Ala Glu Gln Val  
 2355 2360 2365  
 35 Asp Tyr Val Ile Lys Glu Ala Thr Asn Leu Asp Asn Leu Ala Gln Leu  
 2370 2375 2380  
 Tyr Glu Gly Trp Thr Ala Trp Val  
 2385 2390  
 40  
 <210> 6  
 <211> 779  
 45 <212> PRT  
 <213> Homo sapiens  
 <400> 6  
 Met Leu His Glu Leu Asp Gly Leu Ile Glu Gln Thr Thr Asp Gly Val  
 1 5 10 15  
 50

## Claims

1. An isolated human BLIP protein or a variant thereof.
2. An isolated protein having a sequence selected from:

- (a) SEQ ID NO:4; and
- (b) sequences that have at least about 70% sequence similarity to SEQ ID NO:4, and wherein the protein is a BLIP protein.

- 5 3. A kinase inactive mutant of a BLIP protein according to claim 2.
4. A kinase inactive mutant of a BLIP protein having SEQ ID NO:5.
5. An isolated protein having a sequence selected from:
  - 10 (a) SEQ ID NO:6; and
  - (b) sequences that have at least about 90% sequence similarity to SEQ ID NO:6, and wherein the protein is a LIP protein.
- 15 6. An isolated nucleic acid molecule having a nucleotide sequence selected from:
  - (a) a nucleotide sequence encoding a protein of SEQ ID NO:4;
  - (b) a nucleotide sequence that hybridizes to the sequence of (a) above under conditions represented by a wash stringency of 0.3M NaCl, 0.03M sodium citrate, and 0.1 % SDS at 60 degrees C°, and which encodes a BLIP protein;
  - 20 (c) a nucleotide sequence complementary to a sequence of (a) - (b), above.
7. An isolated nucleotide molecule according to claim 6 which is a cDNA sequence.
- 25 8. An isolated nucleic acid which encodes a BLIP protein having the amino acid sequence given herein as SEQ ID NO:4.
9. An isolated nucleic acid molecule having a nucleotide sequence selected from:
  - 30 (a) a nucleotide sequence encoding a protein of SEQ ID NO:6;
  - (b) a nucleotide sequence that hybridizes to the sequence of (a) above under conditions represented by a wash stringency of 0.3M NaCl, 0.03M sodium citrate, and 0.1% SDS at 60 degrees C°, and which encodes a LIP protein with at least about 90% amino acid sequence similarity to SEQ ID NO:6;
  - 35 (c) a nucleotide sequence complementary to a sequence of (a) - (b), above.
10. An isolated nucleic acid which encodes a LIP protein having the amino acid sequence given herein as SEQ ID NO:6.
11. A nucleic acid construct having a promoter and a heterologous nucleic acid operably linked to said promoter, wherein said heterologous nucleic acid is a nucleic acid according to claim 6-10.
- 40 12. A cell containing a nucleic acid construct according to claim 11.
13. A cell containing a nucleic acid construct according to claim 11 and capable of expressing the encoded protein.
- 45 14. An expression vector comprising a nucleotide sequence according to any one of claims 6 to 10, which is capable of expressing the encoded protein.
15. A cell line according to claim 13 which is a modified HEK293 or HeLa cell line.
- 50 16. A method of producing a BLIP protein comprising introducing into an appropriate cell line a suitable vector comprising a nucleotide sequence according to claim 6, under conditions suitable for obtaining expression of the nucleotide sequence.
17. An antibody that specifically binds to a protein encoded by a nucleic acid molecule of claim 6.
- 55 18. An antibody according to claim 17 that is a monoclonal antibody.
19. A method of screening compounds for the ability to modulate BLIP-dependent NFκB activation, comprising ad-

ministering a test compound to a test cell and detecting any increase or decrease in BLIP-dependent NF $\kappa$ B activation in the test cell, compared to a control cell that was not administered the test compound.

20. A method according to claim 19 where said detection of BLIP-dependent NF $\kappa$ B activation utilizes a  $\kappa$ B-dependent reporter gene.

21. A compound identified by the method according to claim 19.

22. A method of inhibiting NF- $\kappa$ B activation in a cell comprising administering to the cell an inhibitor of BLIP-related NF- $\kappa$ B activation, in an amount effective to inhibit NF- $\kappa$ B activation therein compared to the NF- $\kappa$ B activation that would occur in the absence of said inhibitor.

23. A method according to claim 22 wherein said inhibitor is a BLIP kinase-dead mutant or a LIP protein.

24. A method of treatment or prophylaxis of a disorder that is responsive to inhibition of BLIP-dependent NF- $\kappa$ B activation in a mammalian subject, comprising administering to said subject an effective amount of a compound that inhibits BLIP-dependent NF- $\kappa$ B activation.

25. A method according to claim 24 where said disorder is selected from neoplastic, inflammatory and immune disorders.

26. The use of a compound that modulates of BLIP-dependent NF- $\kappa$ B activation in a method of formulating a medicament for treatment or prophylaxis of a disorder that is responsive to modulation of BLIP-dependent NF- $\kappa$ B activation.

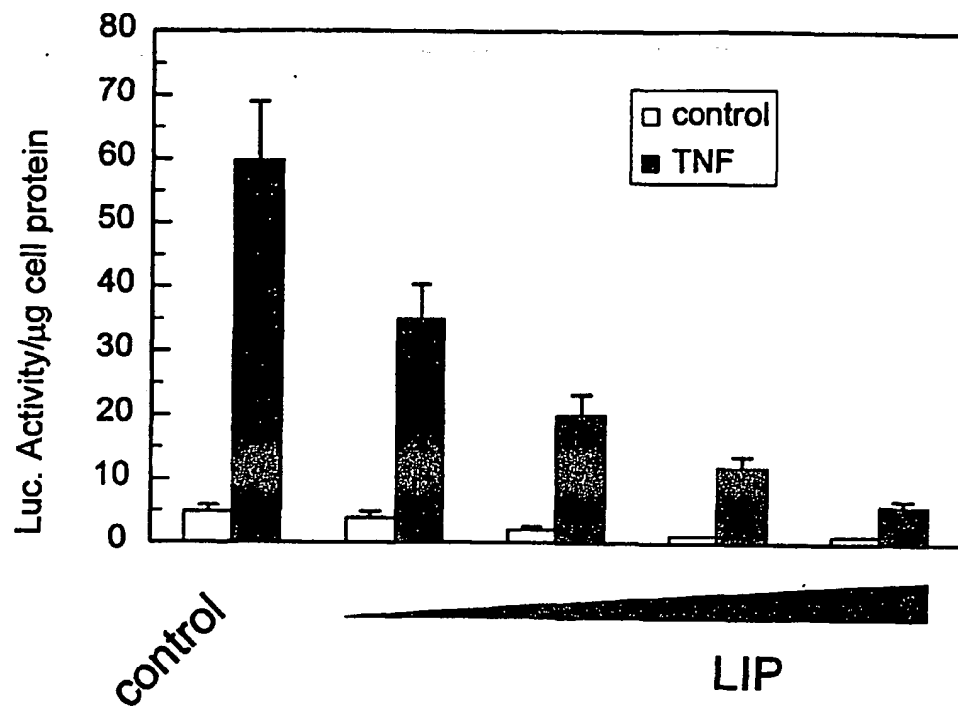


FIG. 1

MKKLLPNMLS	PDPRELQKSI	EVQLLRSSVC	LATALNPIEQ	DQKWQSITEN	VVKYLKQTSR	60
IAIGPLRLST	LTVSQSLPVL	STLQLYCSSA	LENTVSNRLS	TEDCLIPLFS	EALRSCKQHD	120
VRPWMQALRY	TMYNQQLLEK	IKEQTVPIRS	HMELGLTAA	KFARKRGVNS	LATRLLAQCS	180
EVQLGKTTTA	QDLVQHFKKL	STQGOVDEKW	GPELDIEKTK	LLYTAGQSTH	AMEMLSSCAI	240
SFCKSVKAEY	AVAKSILTLA	KWIQAEWKEI	SGQLKQVYRA	QHQQNFTGLS	TLSKNILTIL	300
ELPSVNTMEE	EYPRIEEST	VHIGVGEPDF	ILGQLYHLSS	VQAPEVAKSW	AALASWAYRW	360
GRKVVDNASQ	GEGVRLPRE	KSEVQNLLPD	TITEEEKERI	YGILGQAVCR	PAGIQDEDIT	420
LQITESEDNE	EDDMVDVIWR	QLISSCPWLS	ELDESATEGV	IKVWRKVVD	IPSLYKLSCS	480
AYFTFLKUNA	GQIPLEDDEP	RLHLSHRVEQ	STDDMIVMAT	LRLRLLVKH	AGELRQYLEH	540
GLETTPTAPW	RGIIPLFSR	LNHPEVYVRQ	SICNLLCRVA	QDSPHLILYP	AIVGTISLSS	600
ESQASGNKFS	TAIPTLLGNI	QGEELLVSEC	EGGSPPASQD	SNKDEPKSGL	NEDQAMQDC	660
YSKIVDKLSS	ANPTMVLQVQ	MLVAELRRVT	VLWDELWLG	LLQQHMYVLR	RIQOLEDEVK	720
RVQNNNTLRK	EKIAIMREK	HTALMKPIVF	ALEHVSITA	APAETPHEKW	FQDNYGDAIE	780
NALEKLKTPL	NPAKPGSSWI	PFKEIMLNLO	QRAQKRASYI	LRLEEISPLW	AAMTNTETAL	840
PGEVSARDTV	TIHVGGTIT	ILPTKTKPKK	LLFLGSDGKS	YPYLFKGLD	LHLDERIMQF	900
LSIVNTMFAT	INRQETPRFH	ARHYSVTPLG	TRSGLIQWVD	GATPLFGLYK	RWQQREAALQ	960
AQKAQDSYQT	PQNPQIVPRP	SELYYSKIGP	ALKTVGLSLD	VSRRDWPLHV	MKAVLEELME	1020
ATPNNLLAKE	LWSSCTTPDE	WWRVTQSYAR	STAVMSMGY	IIGLGRHLD	NVLIDMTTGE	1080
VVHIDYNVCF	EKGKSLRVE	KVPFRMTQNI	ETALGVTGVE	GVFRLSCEQV	LHIMRRGRET	1140
LLTLLEAFVY	DPLVDWTAGG	EAGFAGAVYG	GGGQQAESKQ	SKREMERET	RSLFSSRVAE	1200
IKVNWFKNRD	EMLVVLPKLD	GSLDEYLSLQ	EQITDVEKLQ	GKLEELIEFL	EGAEGVDHPS	1260
HTLQHRYSEH	TQLQTQORAV	QEAIQVKLNE	FEQWITHYQA	AFNNLEATQL	ASLLQEISTQ	1320
MDLGPPSYVP	ATAFLQNAQ	AHLISQCEQL	EGEVGALLQ	RRSVLRGCLE	QLHHYATVAL	1380
QYPAIFQKH	RIEQWKTWME	ELICNTTVER	CQELYRKYEM	QYAPQPPPTV	CQFITATEMT	1440
LQRYAADINS	RLIRQVERLK	QEAIVTPVCE	DQLKEIERCI	KVFLHENGEE	GSLSLASVII	1500
SALCTLTRRN	LMMEGAASSA	GEQLVDLTSR	DGAWFLEELC	SMSGNVTCIV	QLLKQCHLVP	1560
QDLDPNPME	ASETVHLANG	VYTSIQELNS	NFRQIIFPEA	LRCLMKGEYT	LESMLHELDG	1620
LIEQTTDGV	LQTLVESLQA	YLRNAAMGLE	EETHAHYIDV	ARLLHAQYGE	LIQPRNGSVD	1680
ETPKMSAGQM	LLVAFDGMFA	QVETAFSLV	EKLKMEIPI	AWRKIDIIRE	ARSTQVNFDD	1740
DDNHRQVLEE	IFFLKRLQTI	KEFPRLCGTF	SKTLGSSSSL	EDQNTVNGPV	QIVNVKTLFR	1800
NSCFSEDQMA	KPIKAFTADF	VRQLLIGLPN	QALGLTLCSF	ISALGVDIIA	QVEAKDFGAE	1860
SKVSVDLCK	KAVEHNIQIG	KFSQLVMNRA	TVLASSYDTA	WKKHDLVRRL	ETSISCKTS	1920
LQRVQLHIAM	FQWQHEDLLI	NRPQAMSVTP	PPRSAILTSM	KKKLHTLSQI	ETSIATVQEK	1980
LAALESSIEQ	RLKWAGGANP	ALAPVLQDFE	ATIAERNLV	LKESQRASQV	TFLCSNIIHF	2040
ESLRTRTAEA	LNLDAALFEL	IKRCQQMCSP	ASQFNSSVSE	LELRLQVRD	TGLEHPIGSS	2100
EWLLSAHKQL	TQDMSTQRAI	QTEKEQQIET	VCETIQNLVD	NIKTVLTGHN	RQLGDKHLL	2160
KAMAKDEEAA	LADGEDVPYE	NSVRQFLGEY	KSWQDNIQTV	LFTLVQAMGQ	VRSEHVEML	2220
QEITPTLKE	KTQSQSIYNN	LVSFASPLVT	DATNECSSPT	SSATYQPSFA	AAVRSNTGQK	2280
TQPDVMSQNA	RKLIQKNLAT	SADTPPSTVP	GTGKSVACSP	KKAVRDPKTG	KAVQERNSYA	2340
VSVWKRKAK	LEGRDVPNR	RMSVAEQVDY	VIKEATNLDN	LAQLYEGWTA	WV	2392

FIG. 2A

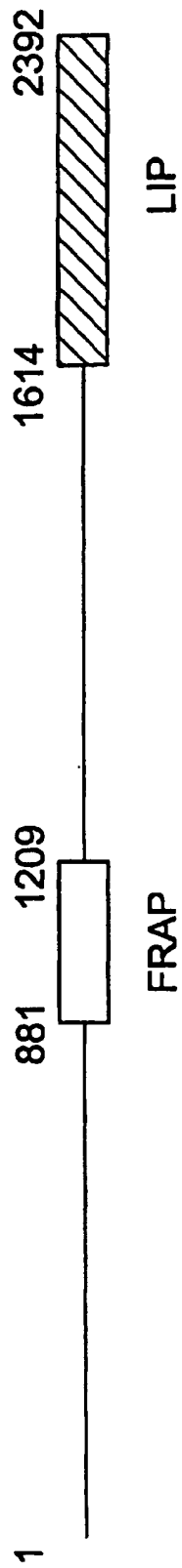


FIG. 2B

```

BLIP 821 LRLEISPLAAMTTEIALPGEVSARDTVT-IHSVGGTITILPTKTKPKLLFLGSDGK
FRAP 2121 LELQYVSPKLLMCRDLELAVPGTYDPNQPIIRIQSIAPSLQVITSKQRPRKLTLMGSNGH
      * * ** * * * * * * * * * * * * * * *
BLIP 880 SYPYLFKGLEDLHLDERIMQFLSIVNTMFATINRQETPRFHARHYSVTPLGTRSGLIQWV
FRAP 2181 EFVFLKKGHEDLRQDERVMQLFGLVNTLLANDPTSLRKNLSIQRYAVIPLSTNSGLIGWV
      * * ** * * * * * * * * * * * * * * *
BLIP 940 DGATPLFGLYKRWQOREAALQAQKAQDSYQTPQNPGIVPRPSELYYSKIGPALKTVGLSL
FRAP 2241 PHCDTLHALIRDYREKK-----ILLNIEHRIMLRMAPDYDHLTLMQ
      * * * * *
BLIP 1000 DVSRDWPPLHVMKAVLEELMEATPPNLLAKELWSSCTTPDEWWRVTQSYARSTAVMSMVG
FRAP 2283 KVE-----VFEHAVNNTAGDDDLAKLLWLKSPSSEVWFDRRTNYTRSLAVMSMVG
      * * * * *
BLIP 1060 YIIGLDRHLDNVLIDMTTGEVVHIDYNVCFEKGKSL-RVPEKVPFRMTQNIETALGVTG
FRAP 2332 YILGLDRHPSNMLDRLSGKILHIDFQDCFEVAMTREKFEKIPFRLTRMLTNAMEVTG
      ** ***** * * * * *
BLIP 1119 VEGVFRLSCEQVLHIMRRGRETLTLLLEAFVYDPLVDW
FRAP 2392 LDGNYRITCHTVMEVLREHKDSVMAVLEAFVYDPLLNW
      * * * * *

```

FIG. 3A

**FIG. 3B**



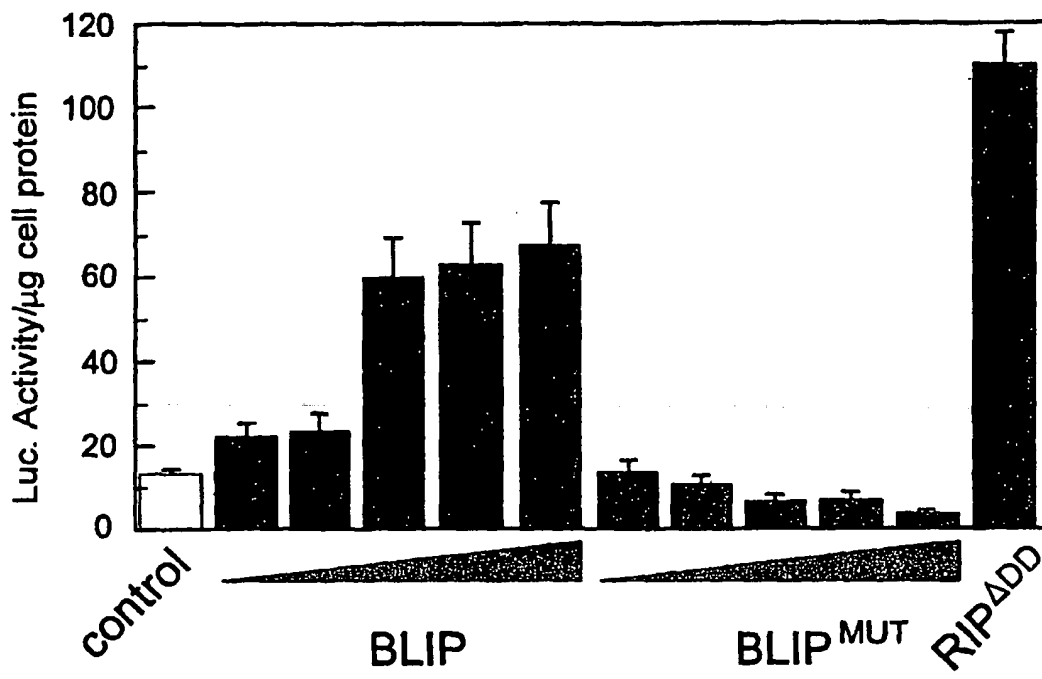


FIG. 4A

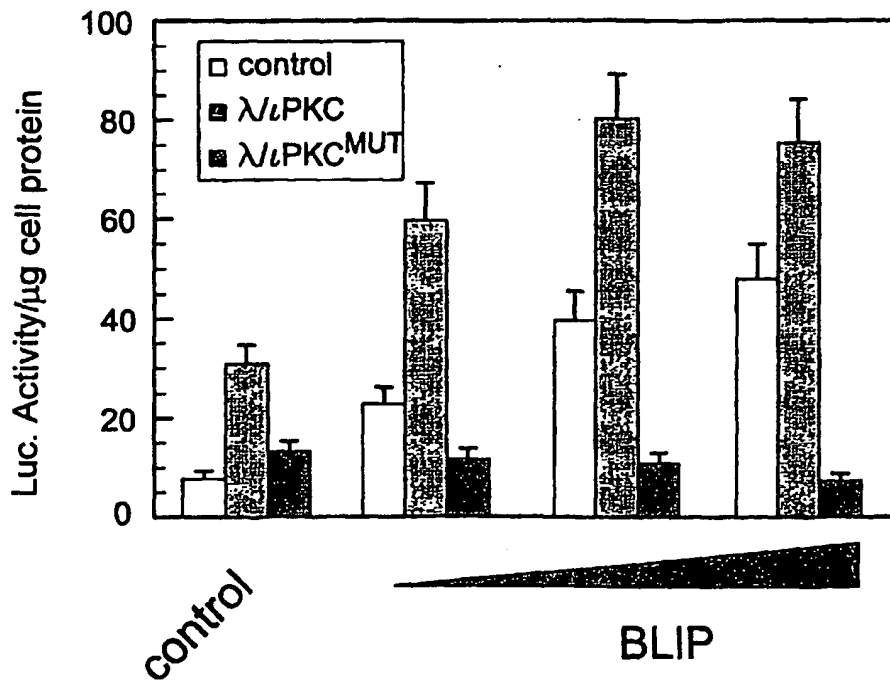


FIG. 4B

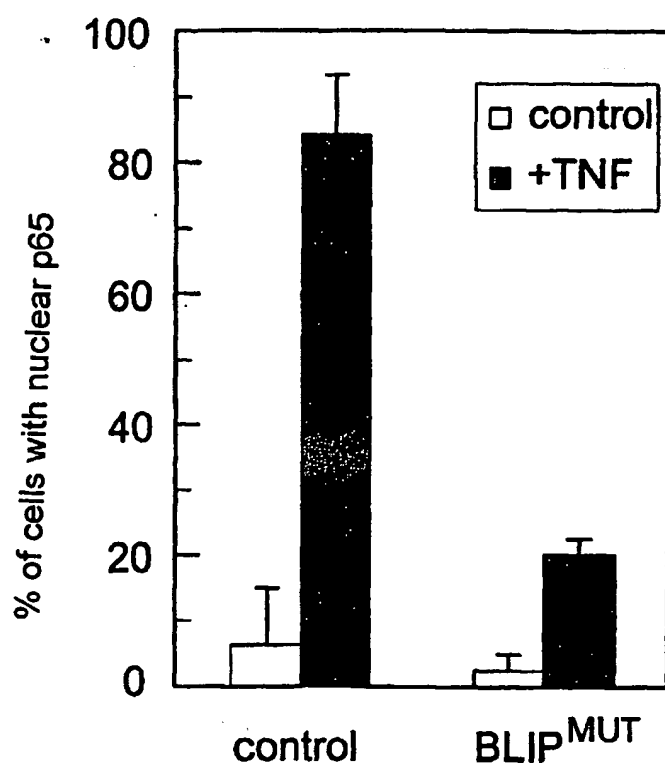


FIG. 5

AGAAAAATA	GAGTTTCACT	CTGTTGCCCA	GGCTGGAGTG	CAGTGACGCA	ATCTCGGCTC	60
ACTGCAGCCT	CTGCCCTCTG	AGTTCAAGTG	ATTCTCCTGC	CTCAGCCTCC	TGAGTAGCTG	120
GGATTACAGG	TGTCTGCCAC	CGTGCCCAGA	CATGAAAAAA	CTGCTTCCTA	ACATGTTAAG	180
TCCGGATCCG	AGGGAAC TTC	AGAAATCCAT	TGAAGTTCAA	TTGTTAAGAA	GTTCTGTTTG	240
TTTGGCAACT	GCTTTAAACC	CGATAGAACA	AGATCAGAAG	TGGCAGTCTA	TAACTGAAAA	300
TGTGGTAAAG	TACTTGAAGC	AAACATCCCG	CATCGCTATT	GGACCTCTGA	GACTTTCTAC	360
TTTAACAGTT	TCACAGTCTT	TGCCAGTTCT	AAGTACCTTG	CAGCTGTATT	GCTCATCTGC	420
TTTGGAGAAC	ACAGTTTCTA	ACAGACTTTC	AACAGAGGAC	TGTCTTATTC	CACTCTTCAG	480
TGAAGCTTTA	CGTTCATGTA	AACAGCATGA	CGTGAGGCCA	TGGATGCAGG	CATTAAGGTA	540
TACTATGTAC	CAGAATCAGT	TGTTGGAGAA	AATTAAAGAA	CAAACAGTCC	CAATTAGAAG	600
CCATCTCATG	GAATTAGGTC	TAACAGCAGC	AAAATTTGCT	AGAAAACGAG	GGAATGTGTC	660
CCTTGCAACA	AGACTGCTGG	CACAGTGCAG	TGAAGTTCAG	CTGGGAAAGA	CCACCACTGC	720
ACAGGATTTA	GTCCAACATT	TTAAAAAACT	ATCAACCCAA	GGTCAAGTGG	ATGAAAAATG	780
GGGGCCCCGAA	CTTGATATTG	AAAAAACCAA	ATTGCTTTAT	ACAGCAGGCC	AGTCAACACA	840
TGCAATGGAA	ATGTTGAGTT	CTTGTGCCAT	ATCTTTCTGC	AAGTCTGTGA	AAGCTGAATA	900
TGCAGTTGCT	AAATCAATTC	TGACACTGGC	TAAATGGATC	CAGGCAGAAT	GGAAAGAGAT	960
TTCAGGACAG	CTGAAACAGG	TTTACAGAGC	TCAGCACCAA	CAGAACTTCA	CAGGTCTTTC	1020
TACTTTGTCT	AAAAACATAC	TCACTCTAAT	AGAACTGCCA	TCTGTTAATA	CGATGGAAGA	1080
AGAGTATCCT	CGGATCGAGA	GTGAATCTAC	AGTGCATATT	GGAGTTGGAG	AACCTGACTT	1140
CATTTTGGGA	CAGTTGTATC	ACCTGTCTTC	AGTACAGGCA	CCTGAAGTAG	CCAAATCTTG	1200
GGCAGCGTTG	GCCAGCTGGG	CTTATAGGTG	GGGCAGAAAG	GTGGTTGACA	ATGCCAGTCA	1260
GGGAGAAGGT	GTTCTGTCTG	TGCCTAGAGA	AAAATCTGAA	GTTTCAAGATC	TACTTCCAGA	1320
CACTATAACT	GAGGAAGAGA	AAGAGAGAAT	ATATGGTATT	CTTGGACAGG	CTGTGTGTCC	1380
GCCGGCGGGG	ATTCAGGATG	AAGATATAAC	ACTTCAGATA	ACTGAGAGTG	AAGACAACGA	1440
AGAAGATGAC	ATGTTTGATG	TTATCTGGCG	TCAGTTGATA	TCAAGCTGCC	CATGGCTTTC	1500
AGAAGTTGAT	GAAAGTGCAA	CTGAAGGAGT	TATTAAAGTG	TGGAGGAAAG	TTGTAGATAG	1560
AATATTCAGC	CTGTACAAAC	TCTCTTGACG	TGCATACTTT	ACTTTCCTTA	AACTCAACGC	1620
TGGTCAAATT	CCTTTAGATG	AGGATGACCC	TAGGCTGCAT	TTAAGTCACA	GAGTGAACA	1680
GAGCACTGAT	GACATGATTG	TGATGGCCAC	ATTGCGCCTG	CTGCGGTTGC	TCGTGAAGCA	1740
CGCTGGTGAG	CTTCGGCAGT	ATCTGGAGCA	CGGCTTGGAG	ACAACACCCA	CTGCACCATG	1800
GAGAGGAATT	ATTCCGCAAC	TTTTCTCAGC	CTTAAACCAC	CCTGAAGTGT	ATGTGCGCCA	1860
AAGTATTTGT	AACCTTCTCT	GCCGTGTGGC	TCAAGATTCC	CCACATCTCA	TATTGTATCC	1920
TGCAATAGTG	GGTACCATAT	CGCTTAGTAG	TGAATCCAG	GCTTCAGGAA	ATAAATTTTC	1980
CACTGCAATT	CCAACTTTAC	TTGGCAATAT	TCAAGGAGAA	GAATTGCTGG	TTTCTGAATG	2040
TGAGGGAGGA	AGTCCTCCTG	CATCTCAGGA	TAGCAATAAG	GATGAACCTA	AAAGTGGATT	2100
AAATGAAGAC	CAAGCCATGA	TGCAGGATTG	TTACAGCAAA	ATTGTAGATA	AGCTGTCCCTC	2160
TGCAAACCCC	ACCATGGTAT	TACAGGTTCA	GATGCTCGTG	GCTGAACTGC	GCAGGGTCAC	2220
TGTGCTCTGG	GATGAGCTCT	GGCTGGGAGT	TTTGCTGCAA	CAACACATGT	ATGTCTTGAG	2280
ACGAATTCAG	CAGCTTGAAG	ATGAGGTGAA	GAGAGTCCAG	AACAACAACA	CCTTACGCAA	2340
AGAAGAGAAA	ATTGCAATCA	TGAGGGAGAA	GCACACAGCT	TTGATGAAGC	CCATCGTATT	2400

FIG. 6

TGCTTTGGAG	CATGTGAGGA	GTATCACAGC	GGCTCCTGCA	GAAACACCTC	ATGAAAAATG	2460
GTTTCAGGAT	AACTATGGTG	ATGCCATTGA	AAATGCCCTA	GAAAAACTGA	AGACTCCATT	2520
GAACCCTGCA	AAGCCTGGGA	GCAGCTGGAT	TCCATTTAAA	GAGATAATGC	TAAATTTGCA	2580
ACAGAGAGCA	CAGAAACGTG	CAAGTTACAT	CTTGCGTCTT	GAAGAAATCA	GTCCATGGTT	2640
GGCTGCCATG	ACTAACACTG	AAATTGCTCT	TCCTGGGGAA	GTCTCAGCCA	GAGACACTGT	2700
CACAATCCAT	AGTGTGGGCG	GAACCATCAC	AATCTTACCG	ACTAAAACCA	AGCCAAAGAA	2760
ACTTCTCTTT	CTTGGATCAG	ATGGGAAGAG	CTATCCTTAT	CTTTTCAAAG	GACTGGAGGA	2820
TTTACATCTG	GATGAGAGAA	TAATGCAGTT	CCTATCTATT	GTGAATACCA	TGTTTGCTAC	2880
AATTAATCGC	CAAGAAACAC	CCCGGTTCCA	TGCTCGACAC	TATTCTGTAA	CACCACTAGG	2940
AACAAGATCA	GGACTAATCC	AGTGGGTAGA	TGGAGCCACA	CCCTTATTTG	GTCTTTACAA	3000
ACGATGGCAA	CAACGGGAAG	CTGCCTTACA	AGCACAAAAG	GCCCAAGATT	CCTACCAAAC	3060
TCCTCAGAAT	CCTGGAATTG	TACCCCGTCC	TAGTGAACCT	TATTACAGTA	AAATTGGCCC	3120
TGCTTTGAAA	ACAGTTGGGC	TTAGCCTGGA	TGTGTCCCGT	CGGGATTGGC	CTCTTCATGT	3180
AATGAAGGCA	GTATTGGAAG	AGTTAATGGA	GGCCACACCC	CCGAATCTCC	TTGCCAAAGA	3240
GCTCTGGTCA	TCTTGACAAA	CACCTGATGA	ATGGTGGAGA	GTTACGCAGT	CTTATGCAAG	3300
ATCTACTGCA	GTCATGTCTA	TGGTTGGATA	CATAATTGGC	CTTGGAGACA	GACATCTGGA	3360
TAATGTTCTT	ATAGATATGA	CGACTGGAGA	AGTTGTTTAC	ATAGATTACA	ATGTTTGCTT	3420
TGAAAAAGGT	AAAAGCCTTA	GAGTTCCTGA	GAAAGTACCT	TTTCAATGA	CACAAAACAT	3480
TGAAACAGCA	CTGGGTGTAA	CTGCAGTAGA	AGGTGTATTT	AGGCTTTCAT	GTGAGCAGGT	3540
TTTACACATT	ATGCGGCGTG	GCAGAGAGAC	CCTGCTGACG	CTGCTGGAGG	CCTTTGTGTA	3600
CGACCCTCTG	GTGGACTGGA	CAGCAGGAGG	CGAGGCTGGG	TTTGCTGGTG	CTGTCTATGG	3660
TGGAGGTGGC	CAGCAGGCCG	AGAGCAAGCA	GAGCAAGAGA	GAGATGGAGC	GAGAGATCAC	3720
CCGCAGCCTG	TTTCTTCTTA	GAGTAGCTGA	GATTAAGGTG	AACTGGTTTA	AGAATAGAGA	3780
TGAGATGCTG	GTTGTGCTTC	CCAAGTTGGA	CGGTAGCTTA	GATGAATACC	TAAGCTTGCA	3840
AGAGCAACTG	ACAGATGTGG	AAAACTGCA	GGGCAACTA	CTGGAGGAAA	TAGAGTTTCT	3900
AGAAGGAGCT	GAAGGGGTGG	ATCATCCTTC	TCATACTCTG	CAACACAGGT	ATTCTGAGCA	3960
CACCCAACTA	CAGACTCAGC	AAAGAGCTGT	TCAGGAAGCA	ATCCAGGTGA	AGCTGAATGA	4020
ATTTGAACAA	TGGATAACAC	ATTATCAGGC	TGCATTCAAT	AATTTAGAAG	CAACACAGCT	4080
TGCAAGCTTG	CTTCAAGAGA	TAAGCACACA	AATGGACCTT	GGTCTCCAA	GTTACGTGCC	4140
AGCAACAGCC	TTTCTGCAGA	ATGCTGGTCA	GGCCCACTTG	ATTAGCCAGT	GCGAGCAGCT	4200
GGAGGGGGAG	GTTGGTGCTC	TCCTGCAGCA	GAGGCGCTCC	GTGCTCCGTG	GCTGTCTGGA	4260
GCAACTGCAT	CACATATGCAA	CCGTGGCCCT	GCAGTATCCG	AAGGCCATAT	TTCAGAAACA	4320
TCGAATTGAA	CAGTGGGAAGA	CCTGGATGGA	AGAGCTCATC	TGTAACACCA	CAGTAGAGCG	4380
TTGTCAAGAG	CTCTATAGGA	AATATGAAAT	GCAATATGCT	CCCCAGCCAC	CCCCAACAGT	4440
GTGTCAAGTT	ATCACTGCCA	CTGAAATGAC	CCTGCAGCGA	TACGCAGCAG	ACATCAACAG	4500
CAGACTTATT	AGACAAGTGG	AACGCTTGAA	ACAGGAAGCT	GTCACGTGTC	CAGTTTGTGA	4560
AGATCAGTTG	AAAGAAATTG	AACGTTGCAT	TAAAGTTTTT	CTTCATGAGA	ATGGAGAAGA	4620
AGGATCTTTG	AGTCTAGCAA	GTGTTATTAT	TTCTGCCCTT	TGTACCTTA	CAAGGCGTAA	4680
CCTGATGATG	GAAGGTGCAG	CGTCAAGTGC	TGGAGAACAG	CTGGTTGATC	TGACTTCTCG	4740
GGATGGAGCC	TGGTTCTTGG	AGGAACTCTG	CAGTATGAGC	GGAAACGTCA	CCTGCTTGGT	4800
TCAGTTACTG	AAGCAGTGCC	ACCTGGTGCC	ACAGGACTTA	GATATCCCGA	ACCCCATGGA	4860

FIG. 6 CONTINUATION

AGCGTCTGAG	ACAGTTCAC	TAGCCAATGG	AGTGTATACC	TCACTTCAGG	AATTGAATTC	4920
GAATTTCCGG	CAAATCATAT	TTCAGAAAGC	ACTTCGATGT	TTAATGAAAG	GGGAATACAC	4980
GTTAGAAAGT	ATGCTGCATG	AACTGGACGG	TCTTATTGAG	CAGACCACCG	ATGGCGTTCC	5040
CCTGCAGACT	CTAGTGGAAT	CTCTTCAGGC	CTACTTAAGA	AACGCAGCTA	TGGGACTGGA	5100
AGAAGAAACA	CATGCTCATT	ACATCGATGT	TGCCAGACTA	CTACATGCTC	AGTACGGTGA	5160
ATTAATCCAA	CCGAGAAATG	GTTTCAGTTGA	TGAAACACCC	AAAATGTCAG	CTGGCCAGAT	5220
GCTTTTGGTA	GCATTCGATG	GCATGTTTGC	TCAAGTTGAA	ACTGCTTTCA	GCTTATTAGT	5280
TGAAAAGTTG	AACAAGATGG	AAATTCCCAT	AGCTTGCCGA	AAGATTGACA	TCATAAGGGA	5340
AGCCAGGAGT	ACTCAAGTTA	ATTTTTTTGA	TGATGATAAT	CACCGGCAGG	TGCTAGAAGA	5400
GATTTTCTTT	CTAAAAAGAC	TACAGACTAT	TAAGGAGTTC	TTCAGGCTCT	GTGGTACCTT	5460
TTCTAAACA	TTGTCAGGAT	CAAGTTCAC	TGAAGATCAG	AATACTGTGA	ATGGGCCTGT	5520
ACAGATTGTC	AATGTGAAAA	CCCTTTTGTAG	AAACTCTTGT	TTCAGTGAAG	ACCAAATGGC	5580
CAAACCTATC	AAGGCATTCA	CAGCTGACTT	TGTGAGGCAG	CTCTTGATAG	GGCTACCCAA	5640
CCAAGCCCTC	GGACTCACAC	TGTGCAGTTT	TATCAGTGCT	CTGGGTGTAG	ACATCATTGC	5700
TCAAGTAGAG	GCAAAGGACT	TTGGTGCCGA	AAGCAAAGTT	TCTGTTGATG	ATCTCTGTAA	5760
GAAAGCGGTG	GAACATAACA	TCCAGATAGG	GAAGTTCTCT	CAGCTGGTTA	TGAACAGGGC	5820
AACTGTGTTA	GCAAGTTCTT	ACGACACTGC	CTGGAAGAAG	CATGACTTGG	TGCGAAGGCT	5880
AGAAACCACT	ATTTCTTCTT	GTAAGACAAG	CCTGCAGCGG	GTTTCAGCTGC	ATATTGCCAT	5940
GTTTCAGTGG	CAACATGAAG	ATCTACTTAT	CAATAGACCA	CAAGCCATGT	CAGTCACACC	6000
TCCCCACGG	TCTGCTATCC	TAACCAGCAT	GAAAAAGAAG	CTGCATACCC	TGAGCCAGAT	6060
TGAAACTTCT	ATTGCGACAG	TTCAGGAGAA	GCTAGCTGCA	CTTGAATCAA	GTATTGAACA	6120
GCGACTCAAG	TGGGCAGGTG	GTGCCAACCC	TGCATTGGCC	CCTGTACTAC	AAGATTTTGA	6180
AGCAACGATA	GCTGAAAGAA	GAAATCTTGT	CCTTAAAGAG	AGCCAAAGAG	CAAGTCAGGT	6240
CACATTTCTC	TGCAGCAATA	TCATTCAATTT	TGAAAGTTTA	CGAACAAGAA	CTGCAGAAGC	6300
CTTAAACCTG	GATGCGGCGT	TATTTGAACT	AATCAAGCGA	TGTCAGCAGA	TGTGTTTCGT	6360
TGCATCACAG	TTTAAACAGT	CAGTGTCTGA	GTTAGAGCTT	CGTTTATTAC	AGAGAGTGGA	6420
CACTGGTCTT	GAACATCCTA	TTGGCAGCTC	TGAATGGCTT	TTGTCAGCAC	ACAAACAGTT	6480
GACCCAGGAT	ATGTCTACTC	AGAGGGCAAT	TCAGACAGAG	AAAGAGCAGC	AGATAGAAAC	6540
GGTCTGTGAA	ACAATTCAGA	ATCTGGTTGA	TAATATAAAG	ACTGTGCTCA	CTGGTCATAA	6600
CCGACAGCTT	GGAGATGTCA	AACATCTCTT	GAAAGCTATG	GCTAAGGATG	AAGAAGCTGC	6660
TCTGGCAGAT	GGTGAAGATG	TTCCCTATGA	GAACAGTGTT	AGGCAGTTTT	TGGGTGAATA	6720
TAAATCATGG	CAAGACAACA	TTCAAACAGT	TCTATTTACA	TTAGTCCAGG	CTATGGGTCA	6780
GGTTCGAAGT	CAAGAACACG	TTGAAATGCT	CCAGGAAATC	ACTCCCACCT	TGAAAGAACT	6840
GAAAACACAA	AGTCAGAGTA	TCTATAATAA	TTTAGTGAGT	TTTGCATCAC	CCTTAGTCAC	6900
CGATGCAACA	AATGAATGTT	CGAGTCCAAC	GTCATCTGCT	ACTTATCAGC	CATCCTTCGC	6960
TGCAGCAGTC	CGGAGTAACA	CTGGCCAGAA	GACTCAGCCT	GATGTCATGT	CACAGAATGC	7020
TAGAAAGCTG	ATCCAGAAAA	ATCTTGCTAC	ATCAGCTGAT	ACTCCACCAA	GCACCGTTCC	7080
AGGAACTGGC	AAGAGTGTTG	CTTGTAAGTCC	TAAAAAGGCA	GTCAGAGACC	CTAAACTGG	7140
GAAAGCGGTG	CAAGAGAGAA	ACTCCTATGC	AGTGAGTGTG	TGGAAGAGAG	TGAAAGCCAA	7200
GTTAGAGGGC	CGAGATGTTG	ATCCGAATAG	GAGGATGTCA	GTTGCTGAAC	AGGTTGACTA	7260
TGTCATTAAAG	GAAGCAACTA	ATCTAGATAA	CTTGGCTCAG	CTGTATGAAG	GTTGGACAGC	7320

FIG. 6 CONTINUATION

CTGGGTGTGA	ATGGCAAGAC	AGTAGATGAG	TCTGGTTAAG	CGAGGTCAGA	CATCCACCAG	7380
AATCAACTCA	GCCTCAGGCA	TCCAAAGCCA	CACCACAGTC	GGTGGTGATG	CAACTGGGGG	7440
CTTACTCTGA	GGAAACCTAG	GAAATCTCGG	TGCACTAGGA	AGTGAATCCC	GCAGGACAGC	7500
TGCACTCAGG	GATACGCCCA	ACACCATGGC	CTGCAACCCC	AGGGTCAAGG	GTGAAGGAAA	7560
GCAAGCTCAC	CGCCTGAACA	CGGAGATTGT	CTTTCTGCCA	CAGAACAGCA	GCAGACGTGT	7620
CGGGAGGTTA	GCTGCGGAAA	GAAATCGGGA	TGCCGCGGAG	CACAGAGTGA	TTTGGAAGTC	7680
CATTCCACCT	GACCCTGTGT	GTACAATCCA	GGAAAAAAC	AAACCCCACT	CAGAAACAGA	7740
GAAACTGGG	GTCGCGAAGA	AATCACAGCC	AAGGAAGATT	TGATGCATTG	AGATTCTCGT	7800
GTAACACTTG	TTGCTTGGCA	ACAGTACTGG	TTGGGTTGAC	CAGTAAGTAG	AAAAAGGCTA	7860
AAGGCTATGC	GATATGAATT	TCAGAAATGG	ACTGAAAATG	GAGAGCTATG	TAACAGATAC	7920
ACTACAGTAG	AAGAACTTAC	TTCTGAAATG	AAGGGAAAAA	AACCACCCCA	TCGTTCCTTA	7980
CTCCTCCCCA	CCACTTACCC	GTTCCCCCTT	TACCTAATCT	AGTAGATTAG	CCATCTTTCA	8040
AATTCACTTT	TATTTTCAGTC	CTTATATTTT	ATATACTTCC	GTCTCGATGC	TGTTAACAAC	8100
TTCTGATAAC	ATGGAAAATT	CAAGGATTGT	TTAAAGGTCT	GATGATCACA	CACAAAATGT	8160
AATTCCGGTT	ATTTAAGTCA	TTTCTGTGAT	TCTATCATGT	ACAGTTTCCA	GAATGTGCAC	8220
TGTGCATTCA	AAAGTAATGA	ATCTAACAGA	CATTTGATTT	AATGTACACT	CCCTTTTGCT	8280
TATAGTGTGC	ATTTTTTTTG	GAGGTCATTC	AAATTTTCCC	TCTTCTGTGA	TAGCTGTAGT	8340
TTCTTTTATA	GAAAGTAGCT	AATCCAGTGT	AATCTTTTAC	CTTTTTAAAA	ACCAAGATAG	8400
AGTATCTATT	AGAGTTTTAC	ATTGTTGATG	ATAGATTAAAC	AATAAAGTGA	TGTTCTGGTG	8460
GAGGTAGACT	GAAATTTTTT	TAATTCAATG	TTTTCAATTT	ATACTTTTAA	TTTACACTTA	8520
GTAAATTAAA	AGTTGTTTAA	TTTACTTGGC	ATTTTAGGAC	ATGTACATGA	AACAGTGAAA	8580
ATGAGATCCA	CCAACATCTT	TTATTAAGTT	CAGTTATTAG	TCTGTGAAGT	GCTTTACTTT	8640
TTGCACAATT	TTAATAGCTT	GCTATTCAGT	AATACATTAT	AGTGAATTCA	TGATCAAGGT	8700
TTCTTTAAAT	TTAGCATTGC	ATTTTCAGTAC	TGACTGTGTA	AGCTAAATTG	CTGATCCAAA	8760
ATAAAAACCC	AGACTAGAAT	AGGGTTCTTA	AAATCAAGTA	TCAATACAAA	ATAGAACACA	8820
ATTAAAATCT	TAATTGTTGG	CTGGGCACAG	TGGCTCACGC	CTGTAATCCC	AGCACTTTGG	8880
GAGGCCGAGG	CGGGCGGATC	ATGAGGTTAG	GAGAGCGAGA	CCATCCTGGC	GAACACGGTG	8940
AAACCCCGTC	TTTACTAAAA	TACAAAAAAA	ATTAGCCGGG	TGTGGTGGCG	GGCGCTGTGA	9000
GTCCAGCTA	CTCGGGAGGC	TGAGGCAGGA	GAATGGCGTG	AACCCAGGAG	GCGGAGCTTG	9060
CAGTGAGCCG	AGATTGTGCC	ACTGCACCTC	AGCCTGGGCA	ACAGAGCTAG	ACTCTGTGTC	9120
AAAAATAAAT	GACTAGAT					9138

FIG. 6 CONTINUATION

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/ES 00/00308A. CLASSIFICATION OF SUBJECT MATTER <sup>6</sup>:

IPC7 : C12N 9/12, 15/54

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 : C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STRAND, WPI, EPODOC, HCAPLUS, MEDLINE, BIOSIS, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARIA T. DIAZ-MECO ET AL. "Lambda -Interactin Protein, a Novel Protein That Specifically Interacts with the Zinc Finger Domain of the Atypical Protein Kinase C Isotype 9/7 and Stimulates Its Kinase activity in Vitro and Vivo". Molecular and Cellular Biology, Jan. 1996, pages 105-114. Cited in the application.	5, 9-15
A		1-8, 16-26
A	LABORATORIO GLAXO WELLCOME-CSIC DE BIOLOGIA MOLECULAR Y CELULAR, "Localization of atypical Protein Kinase C into Lysosome- Targeted Endosomes through Interaction with p62", Molecular and Cellular Biology, May 1998, pages 3069-3080. Cited in the application	1-26
A	BROWNE . J. et al. "A Mamalian Protein Targeted by G1-arresting Rapamycin-Receptor Complex. Nature" (1994) 369: 756-758.	1-26



Further documents are listed in the continuation of Box C.



See patent family annex.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search  
20 December 2000 (20.12.00)Date of mailing of the international search report  
08 January 2001 (08.01.01)Name and mailing address of the ISA/  
European Patent Office  
Facsimile No.Authorized officer  
Telephone No.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/ ES 00/00308

**Box I** Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims 24 and 25 relate to an object which this Authority is not obliged to search, namely: although they relate to a method for treatment of the human or animal body, the search was carried out and was based on the cited effects of the compounds.
2. ☐ Claims 5, 9-15 relate to elements in the international application which do not comply with established requirements in such a way that a meaningful search cannot be carried, more precisely: although seq. ID 16 is not included as such in the description nor in the section dealing with the lists of sequences, it was deduced from seq. ID 14.
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II** Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims: it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)



**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

☐ BLACK BORDERS

☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES

☐ FADED TEXT OR DRAWING

☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING

☒ SKEWED/SLANTED IMAGES

☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS

☐ GRAY SCALE DOCUMENTS

☐ LINES OR MARKS ON ORIGINAL DOCUMENT

☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY

☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**